AUSTRALIAN JOURNAL OF ZOOLOGY

VOLUME 2
NUMBER 3
NOVEMBER 1954

REGISTERED IN AUSTRALIA FOR TRANSMISSION BY POST AS A PERIODICAL

AUSTRALIAN JOURNAL OF ZOOLOGY

Published by the Commonwealth Scientific and Industrial Research Organization, in collaboration with the Australian National Research Council, and under the general direction of the Editorial Board (Dr. N. S. Noble (Chairman), Professor J. S. Anderson, Professor Sir Macfarlane Burnet, Professor L. H. Martin, and Professor J. G. Wood). A medium for the publication of results of original scientific research in zoology with special emphasis on the descriptive phases.

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MELBOURNE

A TEMPERATURE-CONTROLLED PHYSIOLOGICAL COLOUR RESPONSE IN THE GRASSHOPPER *KOSCIUSCOLA TRISTIS* SJÖST. (ORTHOPTERA : ACRIDIDAE)

By K. H. L. KEY* and M. F. DAY*

[*Manuscript received April* 22, 1954]

CONTENTS

Summary

The alpine grasshopper *Kosciuscola tristis* shows a physiological colour change under the control of temperature. Males are a bright greenish blue above about 25°C and a dull near-black below about 15°C. Intermediate shades are developed at intermediate temperatures. A similar, but less marked, change occurs in the female. The colour change in the male was studied with the aid of a special colour chart, which enabled quantitative ratings of colour to be made.

The histology of the integument is described. In the pale phase a dense layer of highly refractive, very small granules occupies the distal portion of the cells of the epidermis; these are underlain by a layer of larger dark brown granules. In the dark phase the position of these layers is reversed and the nuclei are raised above the basement membrane, on which they rest in the pale phase. At intermediate colour shades the granules show transitional distributions. It is concluded that the colour change is brought about by the migration of the two types of granule in opposite directions within the epidermal cells.

The ecology of *K. tristis* in its natural habitat is discussed. On clear days the insects become pale 2-3 hr after sunrise and begin to turn dark again during the late afternoon; the night is spent in the dark phase. The colour follows closely the temperature given by blackened thermometers, but at any given

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temperature it differs from the equilibrium colour developed when that temperature is maintained constant, because of the lag in accommodation to the continuously changing temperature in the field. It is suggested that the colour change may have a thermoregulatory function.

Two undescribed species of *Kosciuscola* show similar colour changes, but these are confined to the face and ventral surface. The same two types of granule are present in the epidermal cells, including those of the dorsal surface, where they are distributed as in the pale phase of *K. tristis* at all temperatures.

I. Introduction

The genus *Kosciuscola* comprises some five species of brachypterous grasshoppers occupying high altitudes in south-eastern Australia. Only one of these species, *K. tristis* Sjöst.,* has been described (Sjöstedt 1933) and that only from the female. The remaining species and the male of *K. tristis* will be described by J. A. G. Rehn in the course of his current revision of the Australian Acridoidea.

Kosciuscola tristis is found above about 5000 ft on the Kosciusko Massif, New South Wales, and the Victorian Alps. In April 1937 one of us encountered a large population at the summit of Mt. Kosciusko (7316 ft) and noted that the undescribed male was of a rather uniform bright greenish blue colour most unusual in the Acrididae. The female was predominantly brown to gray with black markings, often with certain areas dark green, but with none of the blue colour characteristic of the male. Specimens of both sexes were brought alive to Canberra and caged in the laboratory. Upon later inspection it was found that the males had lost their blue colour and become a dark gray. When the cage was placed outside in direct sunlight, the blue colour returned within half an hour, but gave way to gray once more if the cage was shaded. It was clear that a "physiological" colour change, unique in the Acrididae, was involved. All previously investigated instances of colour change in this family have required at least several days for completion of the change and must therefore be assumed to be of the "morphological" type involving the formation or destruction of pigment.

No opportunity for further investigation of the colour change presented itself until 1952, when the present authors undertook to examine its mechanism. Mr. B. B. Given, of the New Zealand Department of Scientific and Industrial Research, kindly provided us with about 25 males of *K. tristis* from Mt. Kosciusko, and these were used in preliminary laboratory experiments. A 3-day visit was then paid to the Kosciusko Massif, in the course of which observations were made on the colour change in relation to the general ecology of the species and further material was collected; this was used in the main series of laboratory experiments. The present paper deals with the more general results of the study, including the ecological and histological observations. The laboratory experiments on conditioning factors and the physiological mechanism are described in a separate paper (Key and Day 1954).

The results of the preliminary experiments indicated that the colour change was conditioned by temperature, the males being nearly black at temperatures

* Identification by K. H. L. Key: specimens deposited in the Division of Entomology Museum, Canberra.

below about 15°C and blue at temperatures above about 25°C. Other environmental factors appeared to be without effect and there was no inherent diurnal rhythm. Upon transfer from one temperature to another, the change at first proceeded rapidly, and then more slowly to equilibrium, which was attained more quickly in transfers to a higher temperature than to a lower.

To enable quantitative estimations of the colour of individual insects to be made and thus permit the calculation of group means, a colour rating chart was developed. The colours of extreme blue and near-black males were matched with water paints, and two intermediate shades were interpolated at apparently equal colour intervals. Each of the four resulting colours was uniformly applied to an area about 4 in. square on a sheet of white card. The squares were arranged in a row from the bluest to the darkest, each adjacent pair being contiguous, i.e. with no intervening white strip, and were given the values 1-4, 1 being the bluest and 4 the darkest. Interpolation by eye between the four colours gave in all seven values that could be used in rating the colour of individual grasshoppers: 1, 1·5, 2, 2·5, 3, 3·5, and 4. The chart was used for colour rating both in the field and the laboratory. Except where the contrary is stated, all ratings were made on the basis of the dorsal surface, or disk, of the pronotum, the insect being held over the chart and moved from one coloured square to another. A characterization of the four colours by the Ridgway terminology will be found in Section III (*a*); an examination of the reliability of the rating method is made by Key and Day (1954). The chart has been deposited in the C.S.I.R.O. library, Canberra.

II. Ecological Observations in the Natural Habitat

The Kosciusko Massif is an elevated plateau (lat. *c.* 36° 30′ S.) in the New South Wales portion of the Australian Alps, rising from about 3000 ft at its eastern limit to over 7000 ft at the highest points on the western edge. The surface of the plateau is undulating, with rounded peaks rising above the general level. The topography has been described by Taylor, Browne, and Jardine (1925) and more recently by Costin (1954).

The climate of the region above 5000 ft is not well known. However, the average annual rainfall at The Chalet, 5740 ft, is given as 89 in. (information from the Commonwealth Bureau of Meteorology), and the Commonwealth and States Snowy River Committee (1950) estimates the rainfall of the highest points as over 120 in. per annum. Mean maximum and minimum temperatures at The Chalet are 48·5 and 30·0°F (9·2 and −1·1°C) respectively (Commonwealth Bureau of Meteorology). Further information on climate is provided by Costin (1954).

The soils have been described by Costin(1950, 1954), and the vegetation by McLuckie and Petrie (1927) and Costin (1954). McLuckie and Petrie divided the region into three zones: the montane zone from 3000 to approximately 5000 ft, the subalpine zone from approximately 5000 ft to the tree-line at 6000-6500 ft, and the alpine zone from the tree-line to the highest elevations. We are concerned here only with the alpine and subalpine zones, since *K. tristis* does not occur below about 5000 ft in this region.

In the subalpine zone, the *Eucalyptus niphophila* Association forms a subalpine woodland on the upper and middle slopes. The herbaceous stratum is dominated by *Poa australis;* a low shrub stratum is variably developed, and when dense practically eliminates the herbaceous stratum. The lower slopes of this zone are occupied by the *Poa australis-Celmisia longifolia* Association, and the valley bottoms, which are waterlogged for most of the year, by subclimax, mainly shrubby, marsh communities. The alpine zone is characterized by the very uniform *Poa australis-Celmisia longifolia* Association, in which the two chief dominants tend to form pure societies in a patchwork distribution. Low, mainly prostrate or cushion shrubs occur very locally, especially in association with granite boulders.

A useful "Map of the Kosciusko Region," at a scale of 1 mile to the inch, was published in 1949 by the New South Wales Department of Lands, and shows all place names mentioned in this paper. Other maps are the map of "Snow Leases and Permissive Occupancies within and adjacent to Kosciusko State Park," published by the same authority in 1945, and the "Reconnaissance Topographic Survey of the Kosciusko Plateau" given by Taylor, Browne, and Jardine (1925).

Earlier observations of one of us showed that *K. tristis* is not numerous below about 6000 ft, i.e. in the subalpine zone, although in the alpine zone it may reach very high densities and constitutes the predominant insect species of the late summer and autumn. Above 6000 ft its only common acridid associates are a second (undescribed) species of *Kosciuscola,* which will be referred to here as "*Kosciuscola* sp. 2," *Phaulacridium vittatum* (Sjöst.),* *Monistria vinosa excelsa* Rehn,* and *Percassa rugifrons* (Stål).* Of these, only the first approaches or equals *K. tristis* in abundance; the two last are much less frequent and are confined to shrubs or areas containing shrubs.

(*a*) *General Account of the Observations*

The authors reached the Massif on March 12. A population of *K. tristis* of approximately one per 1-3 sq. yd. was located on the Cootapatamba Saddle in the late afternoon, *K.* sp. 2 being also present. The sky was heavily overcast, with continuous cloud reaching almost to ground level at times. At 5.10 p.m. an ordinary mercury-in-glass thermometer gave a reading of 8·6°C, and one with the bulb coated with a dull black varnish read 9·7°C. A sling psychrometer recorded a relative humidity of 94 per cent. The vegetation on the Saddle is a typical community of alternating patches of *Poa australis* and *Celmisia longifolia,* both species being at the time hardly more than 3 in. high. *K. tristis* was on the whole more numerous amongst the *Celmisia* than the *Poa,* and more numerous on the wetter low-lying areas than on the lower slopes. The insects were sitting on the plants and were somewhat sluggish, although the males especially were quite capable of jumping when disturbed. The colour of 10 males was rated by means of the chart. All 10 gave the value 4·0.

* Identifications by K. H. L. Key: specimens deposited in the Division of Entomology Museum, Canberra.

On March 13 the weather was fine and clear, and, apart from three brief periods when the sun was obscured by thin cloud, it remained so for the rest of our stay. During the morning an area near The Chalet was selected for more intensive observations on the colour change in relation to environmental conditions. This area is situated on the upper northerly slope of Mt. Stilwell, a high point on Kangaroo Ridge, which latter runs slightly east of north from the Ramshead Range to Charlotte's Pass and thus flanks the Upper Spencer's Creek valley on the west. It is about half a mile from the road at the 26-mile peg. Within this area, observations were made at three closely adjacent sites.

Site 1 was located on the exposed crest of the ridge, which at this point slopes only slightly to the north. The altitude here was determined by an aneroid as 6375 ft. The vegetation consisted of fairly dense *Poa* to an average height of about 6 in., with a small amount of *Celmisia,* and occasional small shrubs little higher than the *Poa.* Groups of granite rocks a few feet high were scattered about, and a few larger ones well over head height. *K. tristis* was comparatively sparse. At this site the main weather recording was done. A thermohygrograph, a maximum-minimum thermometer, and a mercury-in-glass thermometer (*T*1) graduated to tenths of a degree C were placed together, a few inches from the ground, in the shelter of a small group of rocks, which protected the instruments from rain and sun but left them sufficiently exposed to the wind. A similar thermometer (*T*2), and another (*T*3) with blackened bulb, were placed side by side in the sun in an exposed position immediately adjacent to the group of rocks, while another blackened thermometer (*T*4), also in the sun, was located in a more sheltered position in the lee of the rocks. A sling psychrometer was used to give periodical relative humidity readings at a few inches above the ground.

Site 2 was situated about 100 yd east of site 1, perhaps 20 ft below the crest of the ridge, and at the top of the steep east-facing slope above the valley of Upper Spencer's Creek. It was sheltered on the north and west by massive outcropping granite. The vegetation consisted of *Poa* only. A blackened (*T*5) and an unblackened (*T*6) thermometer were placed side by side in the sun at a few inches from the ground. *K. tristis* was rather more numerous than at site 1.

Site 3 was about 300 yd south-east of site 1, and some 50 ft lower, near the summit of a north-facing slope above the valley of Upper Spencer's Creek. It was about 20 yd east of the head of a small gully and about the same distance from the tree-line further to the east. Numerous shrubs, about 1 ft high, and a few rocks covered more than half of the area, with patches of *Poa* and *Celmisia* in between. *K. tristis* was more abundant than at the other two sites, averaging perhaps 2-3 per sq. yd.; *K.* sp. 2 was present in smaller numbers. Two cages containing *Poa* tussocks and twigs from the shrubs were established here in the shelter of a rock and shrubs, and numerous *K. tristis* males from the local population were placed in them during the course of the observations. Thermometers with blackened bulbs were set up in various situations, as follows: *T*7—in the sun, sheltered by a rock; *T*8—in a small shrub; *T*9—in a dense mass of *Richea gunnii*; *T*10—in a dense shrub; and *T*11—in the sun, in a relatively sheltered position almost surrounded by shrubs.

The instruments at all three sites were read as often as practicable between 11.20 a.m. and 6.40 p.m. on March 13 and again between 6 a.m. and 10.50 a.m. on March 14. Colour ratings of males collected at site 3 were made immediately before or after each set of readings at that site. The number rated on any one occasion (never < 10) was limited by the need to restrict the period over which rating extended, so that the mean rating could be correlated with the temperature readings that preceded or followed it. The ratings at 6.40 p.m. on March 13 and 6.15-6.30 a.m. on March 14 were made on males drawn from one of the cages, since it would have been impossible to find even 10 insects within a reasonable time in the depths of the shrubs to which they had retreated. At all other times, material for rating was freshly collected in the open.

TABLE 1

WEATHER DATA FOR SITE 1, AT 6375 FT ON KANGAROO RIDGE, MARCH 13 AND 14

Date and Time	Sunshine	Relative Humidity (%)	Shade Temp. (°C)	*T*2 (°C)	*T*3 (black) (°C)	*T*4 (black) (°C)
13.iii.1952						
9.00 a.m.	Full	—	8·1	—	—	—
11.20 a.m.	Full	64	9·6	12·5	17·5	—
12.35 p.m.	Full	54	10·6	15·0	20·0	20·5
2.25 p.m.	Full	63	11·5	16·5	21·0	11·5*
4-4.30 p.m.	Intermittent thin cloud					
4.30 p.m.	Full	72	10·3	15·4	19·9	10·6*
5.55-6.10 p.m.	Nil (cloud)					
6.26 p.m.	Sunset					
6.31 p.m.	Nil	86	5·8	4·1*	4·2*	5·4*
14.iii.1952						
6.02 a.m.	Sunrise	92	2·0	−1·0*	−1·0*	0·2*
6.02-6.30 a.m.	Thin cloud					
6.45 a.m.	Full	78	4·1	0·9*	1·1*	5·0
8.25 a.m.	Full	41	8·4	12·1	17·7	16·9
10.50 a.m.	Full	42	12·5	19·8	26·6	24·4

Rainfall: nil.
Max. temp., 24 hr: 12·2°C; min. 0·8°C (from max. and min. thermometer).
Max. temp., 24 hr: 12·8°C; min. 0·6°C respectively (from thermograph chart).
*In shadow.

(*b*) *Results*

The instrument readings made at site 1 provide basic weather data applicable to all three sites on Kangaroo Ridge and approximately comparable with Stevenson screen readings. These data are presented in Table 1. The figures for shade temperature are each the mean of three readings which were always very close, namely, those given by the thermograph chart, by the dry-bulb thermometer of the sling psychrometer, and by the thermometer *T*1 resting beside the thermohygrograph. The temperatures in the columns headed "*T*2",

"*T*3", and "*T*4" are those given by the thermometers so designated above. The figures for relative humidity were determined from the psychrometer readings; the readings of the hygrograph differed widely from these and were disregarded. The barometer stood at about 23·8 in. throughout March 13; it was not read on March 14. Throughout the period of the observations there was a breeze from the west to south-west. Variations in its speed produced rapid fluctuations in the readings of all the thermometers, these being greatest in the exposed blackened thermometer *T*3, which at 11.40 a.m. on March 13 was ranging from 15 to 20°C within a few minutes.

It may be seen from the table that the 14th was considerably warmer and drier than the 13th. The maximum shade temperature for the period was about 12·5°C, but this was reached as early as 10.50 a.m. on the 14th, at which time observations were discontinued; a considerable further rise could be expected to occur, because this temperature is at least 3°C higher than that for the corresponding time on the 13th, after which a rise took place to the maximum for that day, given by the thermograph as 11·4°C at 1.30 p.m. The minimum temperature in the rock shelter was about 0·7°C at 5 a.m., but it fell below freezing in the open, since thermometers *T*2 and *T*3 were both reading −1°C at sunrise on the 14th. The blackened thermometers *T*3 and *T*4 gave closely similar readings when both were in the sun, these ranging from 8 to 14°C higher than the shade temperature. Similar values were given by thermometer *T*5 at site 2.

Considering together the paired blackened and unblackened thermometers *T*2 and *T*3 at site 1 and *T*5 and *T*6 at site 2, there are in all 11 pairs of readings at times when both members of a pair were in the sun. The differences between members of a pair ranged from 3·0 to 6·8°C in favour of the blackened thermometer, with a mean difference of 4·8°C.*

The temperature readings of the five blackened thermometers (*T*7-*T*11) placed in different situations at site 3 are given in Table 2. It will be seen that the thermometers *T*7 and *T*11, which were in the open but sheltered from the wind, gave the highest readings as long as they were in the sunshine. The highest reading of all, 32·9°C, was given by *T*7 at 10.40 a.m. on March 14, when the shade temperature reached its highest value for the period; at this time the difference between the shade temperature and the temperature given by *T*7 or *T*11 also reached its maximum value of 20·7°C. Since, as has already been pointed out, the shade temperature probably rose considerably after 10.50 a.m., it is likely that the reading of *T*7, and possibly also the difference, would likewise have considerably exceeded the values just quoted. Within perhaps half an hour after sunset, the relation between the exposed thermometers and those (*T*8-*T*10) placed in shrubby cover of various kinds was reversed, the highest temperature of 8°C being given by *T*9 in the mass of *Richea*—more than 4°C above the higher of the two "exposed" readings. At this time all three of the sheltered thermometers gave higher readings than either of the

* This difference is of the same order as that found between blackened and unblackened thermometers by Kennedy (1939) in the Sudan (*c.* 3°C), and between blackened and silvered thermometers by Swan (1952) on Mt. Orizaba, Mexico (5·9°C).

TABLE 2

MEAN COLOUR RATING AND BEHAVIOUR OF *K. TRISTIS* MALES AT SITE 3 ON KANGAROO RIDGE DURING MARCH 13 AND 14, WITH THE TEMPERATURES GIVEN BY BLACKENED THERMOMETERS IN DIFFERENT POSITIONS, AND SHADE TEMPERATURES FROM SITE 1

Highest available temperatures in bold face

Date and Time	Shade Temp.* (°C)	*T*7 (°C)	*T*8 (°C)	*T*9 (°C)	*T*10 (°C)	*T*11 (°C)	Colour Rating†	S.E.	Behaviour of Insects
13.iii.1952									
12 noon	10·8								
12.15 p.m.	10·9	25·0	17·2	14·0	10·0	**30·5**	1·96(13)	0·13	In open
3.00 p.m.	11·4	12·8‡	10·5‡	15·3	14·0	**22·4**			In open
3.10 p.m.	11·3								In open
4.50 p.m.	9·2	**17·6**	8·1	11·0	11·6	9·6‡	1·75(12)	0·12	In open
5.05-5.30 p.m.	8·2						2·33(20)	0·12	More sluggish from 4.15 p.m. and more ready to burrow into plants
5.30 p.m.	7·2								More sluggish from 4.15 p.m. and more ready to burrow into plants
5.40 p.m. (sunset)	6·9								Few in open (sunlit side of rocks, tops of shrubs)
5.45 p.m.	6·9	**15·8**	6·8	7·3	7·9	5·8			All in shrubs
6.40 p.m.	5·6	1·8	5·2	**8·0**	6·5	3·6	3·40§(10)	0·19	All in shrubs
14.iii.1952									All in shrubs
6.10 a.m. (sunrise)	3·3	1·6	1·0	**3·2**	2·5	−0·5			
6.15-6.30 a.m.	3·9						3·93§(20)	0·04	All in shrubs
6.35 a.m.	4·2	**5·4**	1·6	4·0	2·9	1·3‡			All in shrubs
7.00 a.m.	6·7	**13·0**	3·3	3·0	4·1	5·8			
7·15¶-7·50 a.m.	8·1						3·18(20)	0·10	Many basking on tops of shrubs: reacted by jumping
c. 8.10 a.m.	9·4	**26·3**	7·9	5·2	5·3	24·0			Mostly still on shrubs: some in open
10.15 a.m.	11·4	**31·3**	11·6	11·4	7·8	30·0			In open
10.25-10.37 a.m.	11·9								In open
10.40 a.m.	12·2	**32·9**	11·0	11·8	8·2	30·4	1·90(20)	0·09	In open
									In open

*From thermograph at site 1. †Numbers rated in brackets. ‡In shadow. §From caged insects, since practically none could be found in the open. ¶This time may be up to 20 min too early, since the timepiece stopped and at 8.15 a.m. was reading 7.55 a.m.

exposed ones. By sunrise on March 14 the reversed relation still obtained, but the earlier relation was restored within about half an hour. An hour after sunset the temperature in the *Richea* was still more than 2°C above the shade temperature at site 1. By sunrise it had fallen to equality with the shade temperature, and it then rose more slowly, not equalling the latter once more until 10.15 a.m., by when it was almost 20°C below the temperature in the sun.

The behaviour of *K. tristis* at site 3 (see Table 2) showed the type of relation to environmental temperatures that is usual in Acrididae. During the greater part of the daylight hours both sexes were to be found in the sunshine, mainly on the ground (especially near the base of *Poa* tussocks in sheltered situations) or sitting on the tussocks. They were comparatively inactive, occasionally feeding on the *Poa* or moving slowly for distances of a foot or so. Groups of some half dozen would often form in favoured situations. The behaviour was on the whole that of "basking." No evidence of withdrawal into shade or exposure to wind was noted, although special attention could not be given to the behaviour aspect and it may have occurred to a limited extent; it was certainly not a marked or general behaviour pattern. In spite of the general inactivity, disturbance by the observer resulted in vigorous jumping, especially on the part of males; disturbed insects kept to the open and only burrowed into shrubs if hard pressed. From about 4.15 p.m. on March 13 the insects became progressively more sluggish and more inclined to burrow into plants when disturbed. The shade temperature at this time was in the vicinity of 9°C and the temperature of a blackened thermometer in sunshine about 17°C. By 5.30 p.m. there were few insects in the open, and these were concentrated on the sunlit side of rocks and to a less extent on the tops of the shrubs. By sunset at 5.40 p.m. all had retreated into the shrubs, the last to be seen in the open being males. The temperature of an exposed blackened thermometer was then still about 16°C, and the shade temperature and the temperature in the shrubs about 7°C.

For at least half an hour after sunrise on March 14, during the greater part of which time the sun was behind thin cloud, the grasshoppers remained in the shrubs. By 7 a.m. many, especially males, had emerged from the shrubs and were basking on their tops and on *Poa* tussocks, keeping their long axis at right angles to the sun's rays. They were very reactive to the approach of the observer at a distance of 4-5 ft, making jumps of 3-4 ft in length. The blackened-bulb temperature was then about 13°C, the shade temperature 6.7°C, and the temperature in the shrubs 4°C or lower. During the following hour most of the insects remained basking on the tops of the shrubs, but there was a progressive desertion of this position, and by 8.10 a.m. the majority had taken up positions at ground level again, under a blackened-bulb temperature up to 26·3°C and a shade temperature of 9·4°C. At 6.10 a.m. the insects in the cages were mainly sheltering underneath and within the *Poa* tussocks that had been placed there. Although the temperature in the cage could not have exceeded about 3·3°C (Table 2), some of them responded to the approach of the observer by jumping.

The mean colour ratings of samples of *K. tristis* males at site 3 are recorded in Table 2 and Figure 1. Figure 1 also includes the rating at Cootapatamba Saddle on March 12. It may be seen that at about sunrise the insects were almost maximally dark. The rating fell quite rapidly during the morning, a perceptible blueing being already apparent by 7 a.m., and reached a figure of 1·75 during the mid afternoon. It rose again during the late afternoon, reaching about 2·8 by sunset and 3·4 by 6.40 p.m. It presumably continued to rise, during the night, up to the sunrise value of almost 4. At Cootapatamba Saddle, where the weather was probably cold and overcast throughout March 12, the rating may never have departed appreciably from 4 on that day.

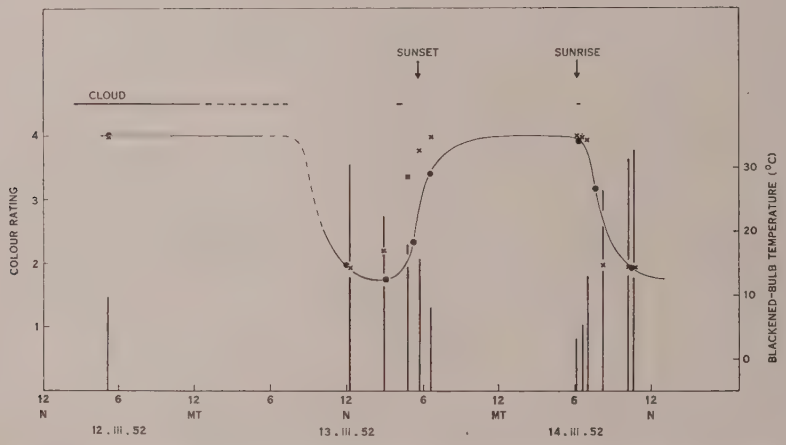

Fig. 1.—Progression of the mean colour rating (solid circles) of samples of *K. tristis* males at site 3 on Kangaroo Ridge and at Cootapatamba Saddle, in relation to sunshine and the highest available temperature (vertical columns), as given by blackened thermometers. The equilibrium rating corresponding to each temperature (see Key and Day 1954, Fig. 7) is given by crosses.

It will be seen from Table 2 that in the evening all the insects at site 3 had retreated into the shrubs at a time when the temperature of exposed blackened thermometers was still, at 16°C, considerably higher than the temperature in the shrubs,* and also higher than the blackened-bulb temperature of 13°C at which emergence from the shrubs took place the following morning. On the other hand, the shade temperatures at the times of retreat and emergence were practically the same, namely, about 7·0 and 6·7°C respectively. It is possible, however, that retreat into and emergence from the shrubs is not determined directly by temperature or a temperature gradient, but by light (cf. Kennedy 1939). Facilities were not available for measuring the internal temperature of the grasshoppers. However, it is likely that this lay in the vicinity of the temperature given by a blackened thermometer exposed in the

* This temperature was measured at a considerable depth in the shrubs. It may have been higher near their surface.

situation occupied by the insect. Thus Kennedy (1939) found that the internal temperature of even a green nymph of *Schistocerca gregaria* (Forsk.) was actually 0·5-2°C *higher*, on the average, than the temperature of a similarly exposed blackened thermometer. Parry (1951), also, concludes that the "temperature of the living animal [terrestrial arthropod] in a given situation is likely to be very similar to that of an inanimate body of the same approximate size, shape, colour and orientation." The bulbs of the thermometers used were approximately the size and shape of a *K. tristis* male. If we may suppose, further, that the darker the insect the higher will its internal temperature tend to be when exposed to the sun (Section V), the internal temperatures at retreat into and emergence from the shrubs may have been less different than the blackened-bulb temperatures of 16 and 13°C would suggest, since at retreat the mean colour rating was 2·8, as against 3·9 at emergence.

Whatever the mechanism governing retreat and emergence may be, and despite the difficulty represented by the higher blackened-bulb temperature in the open at the time of retreat, it is clear that, at each of the times when ratings were made, the insects were occupying the type of situation having the *highest* blackened-bulb temperature of those available to them. This temperature, therefore, has been used for correlation with the colour rating, the probable difference in the absorption of radiation by blue and dark insects being ignored, and it is this temperature that is recorded in Figure 1. Where appropriate, the value employed is the mean of the highest values recorded in the readings made immediately before and immediately after a colour rating.

If the ratings at comparable temperatures in the early morning and evening are compared from Table 2 or Figure 1, it will be seen that the morning ratings are considerably higher. This would seem to be readily explained on the basis that in the morning the rating is declining from an initially high value and the insects have not had time to accommodate to the continuously rising temperature. In the evening the rating is rising from an initially low value, and again it may be assumed that at any given time accommodation to the current (falling) temperature will not have been complete. That is, there is inevitably a lag between temperature change and colour accommodation. It may be noted that the assumed difference in radiation absorption between blue and dark insects would restrict this tendency to a lag, since the internal temperature of the dark insects in the morning would be higher, for a given blackened-bulb temperature, than that of the bluer insects in the evening, and this would tend to lower the rating of the former in comparison with the latter.

The crosses in Figure 1 represent the equilibrium (i.e. end-point) colour ratings which the laboratory experiments described by Key and Day (1954) indicate would be attained at each of the observational temperatures, if those temperatures were maintained constant for some 16-18 hr. It may be seen that these points lie along a curve very similar indeed to the curve of field ratings, but slightly out of phase with it. In the afternoon and evening, when the temperature was falling, the field rating was considerably below the equilibrium value, while in the morning, with rising temperature, it was above the equilibrium value, although not so markedly nor for so long a period. This

fully supports the assumption of a lag in accommodation in the field. The lag approximates fairly consistently to 2 hr, i.e. it would require a further 2 hr at any given temperature in the field for accommodation to be complete; within that time, of course, the temperature has changed.

After noon, at which time the two curves cross, the field rating appears to continue to fall slightly for about 2½ hr, in spite of a substantial fall in temperature. However, the difference between the ratings at noon and 3.10 p.m., upon which this apparent fall rests, is far from significant (see Table 2). It would, indeed, be surprising if any appreciable lag occurred in the reversal of the direction of the colour change upon reversal of the direction of the change in temperature, for Bodenheimer *et al.* (1929) and Koidzumi (1934-35) have found that the internal temperature of an acridid can adjust itself to the air temperature in 6-12 min, and the adjustment of the temperature of the integument is likely to be more rapid than this. The lowest rating given by the field curve is about 0·15 rating units below the minimum of the equilibrium curve, a difference which is again far from significant (see Table 2). In view of the lag between field rating and temperature during the morning, the equilibrium rating would be expected to have a minimum below that of the field rating, and the lack of any significant difference certainly does not suggest that the blackened-bulb temperature is an over-estimate of the internal temperature, even of markedly blue insects, and would be consistent with Kennedy's (1939) findings that internal temperature was on the average above the blackened-bulb temperature. It is of interest to note that the shade temperature, which never exceeded 12·2°C during the period of the observations (see Table 2) would not have permitted any appreciable departure from the maximally dark condition (see Key and Day 1954, Fig. 7).

Before sunrise on March 14, while one observer was on Kangaroo Ridge the other ascended the Etheridge Range. This range, lying immediately south of the road between the 29- and 30-mile pegs (see "Map of the Kosciusko Region"), runs in a north-easterly direction from the Cootapatamba Saddle. Here certain observations on behaviour and colour were made which are consistent, so far as they go, with the more detailed observations on Kangaroo Ridge. On the way up, most of the grasshoppers were out of sight. Some were located in little crevices among small rocks, but a few were seen, quite immobile, high up on large granite boulders, where they had presumably climbed to catch the last rays of the setting sun and then been immobilized by the rapid fall in temperature after sunset. All the males appeared maximally dark. On the easterly side of the crest all the insects seemed to be under cover, and those seen were also maximally dark. Shortly after sunrise the grasshoppers on the easterly slope and the crest emerged from their hiding places in large numbers and exposed themselves to the sun on the tops of shrubs and the easterly faces of boulders. On two large granite outcrops they reached an estimated density of 50 per sq. yd. in places. Later they descended to ground level and dispersed. By 7.30 a.m. the males on the easterly slope were already turning blue, while those on the westerly slope, which had not yet been reached by the sun, were still dark. At 6.30 a.m. on the crest, an unblackened thermo-

meter in the shade at ground level read 2·5°C, and the same reading was given in a *Poa* tussock. At 7.30 a.m. the temperature in the tussock was 8·0°C, and at ground level in the sun 11·0°C.

In spite of the prominent part played by shrubs and rocks in the daily behaviour regime of *K. tristis* at most of the sites where observations were made, it is not yet clear whether these components of the environment have any important role in relation to survival of the insects and hence to the population that any given site can support. Shrubs are most numerous within the subalpine woodland and the lower levels of the alpine zone, and in seral communities in valley bottoms, particularly at the lower levels. On the other hand, *K. tristis,* as has already been indicated, is most numerous in the alpine zone, and seems to be primarily an insect of the *Poa australis-Celmisia longifolia* association. At the summit of Mt. Kosciusko, where a dense population was noted in 1937, there are abundant rocks but very few shrubs. At site 3 on Kangaroo Ridge there were abundant shrubs and a few rocks; on the Etheridge Range abundant rocks, with some associated shrubs, at the points where *K. tristis* was most numerous; and on the Cootapatamba Saddle neither rocks nor shrubs.

Nevertheless, one would expect the principle that a mixed environment is in general more favourable than a uniform one (see Key 1945, p. 115) to apply to this case, and shrubs (presumably also rocks) offer certain evident advantages to the grasshopper. In the first place, they mitigate the severity of the daily minimum temperature (see Table 2). The amelioration may be considerably greater than indicated in the table during the earlier and later parts of the season, when the minimum would be lower than in mid March. Secondly, both shrubs and rocks provide shelter from the wind, and in their lee much higher blackened-bulb temperatures can be attained in the sunshine than in exposed, windy places. Thirdly, both shrubs and rocks provide vantage-points from which grasshoppers can intercept the first and the last rays of the sun in the morning and evening. Even a small survival advantage conferred in these ways could be of great importance in very unfavourable seasons, and a comparatively sparse distribution of shrubs or rocks might be sufficient to provide it. In the habitat of *K. tristis* an environmental factor that must frequently cause mortality is a shorter-than-average growing season, which could result in the death of the majority of the population before it had been able to reproduce. Thus any factor which, by enabling a high average internal temperature to be maintained, can promote speedy development, could be expected to become crucial, from time to time, in determining the general population level and the spatial distribution of population density.

The observations on the Kosciusko Massif established that the colour of *K. tristis* males in the natural habitat is closely correlated with the behaviour of the insects, and especially with the conditions of temperature and insolation upon which both colour and behaviour are dependent. In Section V some suggestions will be made regarding a possible biological role for the colour change that will take these relations into account.

K. sp. 2 was present in varying numbers at all sites where *K. tristis* was noted. Although very little attention could be devoted to it, its behaviour appeared to be in general closely similar to that of *K. tristis.*

III. The Integument in the Pale and Dark Colour Phases*

(*a*) *General Description of the Coloration*

The following descriptions of the colour of *K. tristis* at the two extremes corresponding to warm and cold conditions are based on a few insects maintained at 4·4°C and 35°C for the period necessary to ensure full accommodation. In the male the change is from an almost uniformly near-black insect to an almost uniformly greenish blue insect. In the female there is a clearly marked, somewhat variable pattern, and in addition there are two distinct colour forms, probably under genetic control, in one of which the pronotal disk and certain other parts are green at the higher temperatures. Blue is virtually absent in the female, the colour change on passing from warm to cold conditions consisting of a general darkening and a replacement of the green by near-black. A small percentage of the males used in the laboratory experiments were distinctly greener than average in the pale phase. These gave rise to some difficulty in determining the colour rating; they may correspond genetically to the green form in the female. The typical coloration of the male at the two extremes is illustrated in Plate 1.

(i) *The Male*

Dark phase (*Plate* 1, *Fig.* 1).—Head black, except for the clypeus and mouth-parts, but including the mandible. Clypeus black in the upper part; remainder of clypeus and the mouth-parts (except for the black mandible) shading through a narrow zone of brown dorsally to faintly bluish straw ventrally. Antenna dull olive to olive-brown on all faces. Eye black. Pronotum black throughout, the anterior pale spot on the lateral lobe very faintly lighter than the rest. Meso- and metanotum and abdomen black or faintly indigo-black on the dorsal surface, becoming dark gray-brown towards the lower margins of the tergites, and the abdominal segments with the posterior margin faintly bluish white. Mesometasternum dark indigo-brown; abdominal sternites medium to light indigo-brown. Hind femur with the dorsal surface dull olive; ventral surface pale yellow-brown to dull yellow ochre, sometimes with a very

* When Uvarov (1921) selected the term *phase* by which to designate the forms assumed by certain species of locusts according to whether they had lived a gregarious or non-gregarious existence (see also Key 1950), he was employing a term that had already had a long history of usage in designating a variety of transient states in physics, chemistry, biology, and other branches of science. With the great expansion of research on the phases of locusts in Uvarov's sense, "phase," at least as applied to the Acrididae, has tended to be understood in that sense only. In other groups of insects this has not happened, and "phase" has been available as an extremely useful term for transient states of various kinds. In order to restore this term to its original more general availability within the Acrididae, and at the same time to specify the type of phase meant when the term is used in Uvarov's sense with reference either to Acrididae or to other groups such as Lepidoptera, in which "seasonal" phases, for example, are recognized, some qualifying epithet is required for use with "phase" when Uvarov's sense is intended. The term *kentromorphic,* from the Greek κέντρον, a goad or stimulus, and μορφή, a form, is proposed for this purpose. It emphasizes that the "phase" of Uvarov is a physical *form,* typically a function of *stimulation.*

There is no doubt that the word "phase," in its general sense, is by far the most apt for indicating the temporarily pale and dark conditions of *K. tristis.*

faint olive tinge; external and internal faces light yellow-brown with an olive tinge. Hind tibia and anterior and middle legs essentially as hind femur on their dorsal and ventral surfaces. Tegmen straw to pale olive green above, medium to dark olive green laterally, with the fine venation faintly paler.

In the terminology of Ridgway (1912), the colour of the pronotal disk varies from "greenish slate-black" to "blackish green-gray", although many individuals would be classified simply as "black".

Blue phase (*Plate* 1, *Fig.* 2).—Head deep prussian blue above, pale blue with grayish markings on the gena, pale blue on the face and mandible. Clypeus and mouth-parts (except the mandible) white with a very faint greenish blue tinge. Antenna medium to light olive-brown. Eye black. Pronotum prussian blue on the disk and for the most part on the lobe, but with a distinct black area around the second transverse sulcus on the lobe, and with pale spots on the lobe the same colour as the mouth-parts. Mesonotum and distal 2–3 abdominal segments prussian blue dorsally, metanotum and the remaining abdominal segments a slightly lighter, more greenish blue; lower margin of tergites shading into very pale blue, posterior margins narrowly bluish white. Mesometasternum and abdominal sternites very pale blue. Legs pale yellowish olive on dorsal surfaces, otherwise practically as in the dark phase. Tegmen practically as in the dark phase.

The colour of the pronotal disk is in the vicinity of the "terre-verte", "Montpellier green", "dark goblin blue", and "deep bluish gray-green" of Ridgway.

In the course of the change from the dark to the blue phase the under surface and the face are the parts most rapidly affected. The sides of the body and the dorsal surface of the meso- and metanotum and abdomen change more slowly, and the dorsal surface of the head and pronotum more slowly still. Thus, after only 15 min at 35°C, an originally maximally dark male transferred from 4·4°C was described as follows:

Head dark bluish gray above, lighter on the gena, *pale gray-blue* on the face. Antenna medium dull gray-brown. Pronotum dark bluish gray on the disk, lighter on the lobes, where the black area around the second sulcus is distinct and the pale spots lightly differentiated in bluish cream. Meso- and metanotum and abdomen greenish gray-blue dorsally, becoming pale gray-blue towards the lower margins of the tergites. Mesometasternum and abdominal sternites *very pale gray-blue.*

Thus after 15 min the colour of the face and under surface was almost that characteristic of warm conditions, notwithstanding that the internal temperature of the insect must have been in the vicinity of 4·4°C at the start of the period.

Differences between the colour of head and abdomen and of pronotum and abdomen of males, in terms of rating values, are given by Key and Day (1954).

(ii) *The Female: Green Form*

Dark phase.—Head very dark brown above, dark brown on face, medium brown on gena. Clypeus and mouth-parts mainly pearly gray, tinged with brown in part, especially towards dorsal margin of clypeus. Eye black. Pronotum very deep olive on the disk, almost black; lateral carina brown, bordered in part

with black ventrally; lateral lobe very deep olive, with a longitudinal black bar in the centre of the prozona, bounded ventrally by two pale spots situated anterior and posterior to the second transverse sulcus respectively. Meso- and metanotum mainly dark brown. First abdominal tergite deep olive medially, narrowly black towards the sides. Remainder of abdomen with a deep olive to dark brown dorsomedian stripe, not sharply demarcated, and bounded on either side by a buff stripe bearing numerous small brown dots; the latter stripe bordered laterally by a broad black stripe extending down the lateral portions of each tergite and gradually passing into fawn towards its ventral margin. Mesometasternum and abdominal sternites fawn. A narrow, faintly bluish band occupies the posterior margin of each abdominal tergite and sternite, but is less evident towards the anterior and posterior extremities of the abdomen. Hind femur with the dorsal, external, and internal faces dull yellow-brown, with a suggestion of an olive tinge; ventral face dull chrome yellow. Hind tibia fawn. Anterior and middle legs varying shades of dull yellow-brown to dull chrome yellow.

The colour of the pronotal disk is close to the "olivaceous black (1)" of Ridgway.

Pale phase.—Head olive green above, with a pattern of dark gray-brown markings; fastigial margins buff; gena a network of straw and olive; face pale bluish green. Clypeus and mouth-parts very pale bluish green. Eye black. Pronotum green on the disk; lateral carina mainly buff, bordered in part with black ventrally; lateral lobe green, with longitudinal bar fragmented at the second transverse sulcus, the anterior pale spot straw, the posterior one greenish straw. Meso- and metanotum a slightly yellowish green. Abdomen with a yellow-green dorsomedian stripe, not sharply demarcated, and bounded on either side by a more yellowish green; the latter bounded laterally by a black stripe, which on each tergite is broadest anteriorly and narrows posteriorly, not reaching the posterior margin; below and behind this stripe on each tergite, and less markedly along the whole of the posterior margin of the tergite, very pale slate blue. Mesometasternum and abdominal sternites very pale bluish green. Hind femur with the dorsal surface pale olive green; external face pale olive brown, with the herring-bone ridges very pale greenish; ventral face dull chrome yellow. Anterior and middle legs dull yellow-brown on dorsal and outer faces, with a faint olive tinge; dull chrome yellow on the ventral face.

The colour of the pronotal disk is close to the "spinach green", "grass green", "forest green", and "deep dull yellow-green" of Ridgway.

(iii) *The Female*: *Brown Form*

Dark phase.—Head very dark brown throughout, except lower part of clypeus and mouth-parts, which are fawn to brownish fawn. Eye black. Pronotum very dark brown on the disk, not quite as dark as the dorsal surface of the head; lateral lobe very dark brown, with a broad black bar on the prozona extending from the third sulcus to the anterior margin immediately below the lateral carina, not reaching the anterior margin more ventrally, and extending ventrally to below the anterior pale spot; posterior pale spot scarcely discernible.

Meso- and metanotum and first abdominal tergite dark brown dorsomedially, black towards the sides. Remainder of abdomen with a dark brown dorsomedian stripe bounded on either side by a medium brown stripe bearing small dark brown dots; the latter stripe bordered laterally by a broad black stripe extending down the lateral portions of each tergite and gradually passing into medium brown towards its ventral margin. Mesometasternum medium brown; abdominal sternites fawn with numerous light brown dots. A narrow straw band occupies the posterior margin of each abdominal tergite and sternite, but is less evident towards the anterior and posterior extremities of the abdomen. Hind femur with the dorsal, external, and internal faces dull yellowish brown, with a suggestion of an olive tinge (slightly darker than in green form). Other legs essentially as in green form.

The colour of the pronotal disk is very close to the "bone brown" of Ridgway.

Pale phase.—Head medium gray-brown, with a medial dark gray-brown stripe; fastigial margins buff; gena a network of medium gray-brown and pinkish straw; face buff. Clypeus and mouth-parts very pale blue-gray, with pink overlay in places, especially on part of labrum. Eye black. Pronotum medium gray-brown on the disk; lateral lobe medium gray-brown, the black bar on the prozona as in the dark phase, the pale spots and an area near the anterior margin pinkish buff. Abdomen with a buff dorsomedian stripe, not sharply demarcated, and bounded on either side by somewhat paler buff; the latter bounded laterally by a black stripe, which on each tergite is broadest anteriorly, where it reaches the ventral margin, and narrows posteriorly; below and behind this stripe on each tergite, and less markedly along the posterior margin of the tergite almost to the top of the black stripe, very pale bluish straw, with a pinkish tinge anteriorly. Mesometasternum very pale blue-gray with a pink tinge; abdominal sternites faintly greenish straw. Hind femur with the dorsal surface pale olive brown; external face brown, with the herring-bone ridges narrowly buff; ventral surface dull chrome yellow. Anterior and middle legs dull yellow-brown on dorsal face, dull chrome yellow on ventral face.

The colour of the pronotal disk is close to the "mummy brown", "sepia", and "brownish olive" of Ridgway.

It should be noted that the colour of the dark phase, especially in the male, is essentially dull, or "matt"; this is not a specially noticeable feature of the pale phase.

The four colours of the rating chart may be characterized by the Ridgway terminology as follows:

Grade 1—very close to "Sorrento green".

Grade 2—very close to "myrtle green", or between "prussian green" and "invisible green".

Grade 3—close to "dusky bluish green", or between "dusky dull green" and "dusky dull bluish green".

Grade 4—very close to "dull greenish black (2)", or between that and "olivaceous black (3)".

(*b*) *Histology*

Individuals of both sexes of *K. tristis*, representing various stages in the change from the dark to the pale phase, were fixed by injection with alcoholic Bouin's fluid, the males having been previously rated by means of the chart. Sections 10 μ thick were cut of the posterior portion of the pronotal disk (which has the advantage of being devoid of muscle attachments), the abdomen, and certain other parts. Material to be sectioned was dehydrated with alcohol in the usual way and mounted in canada balsam from xylol. Thus pigments soluble in, or destroyed by, alcohol or fat solvents would be largely or wholly removed from the preparations (see Section V).

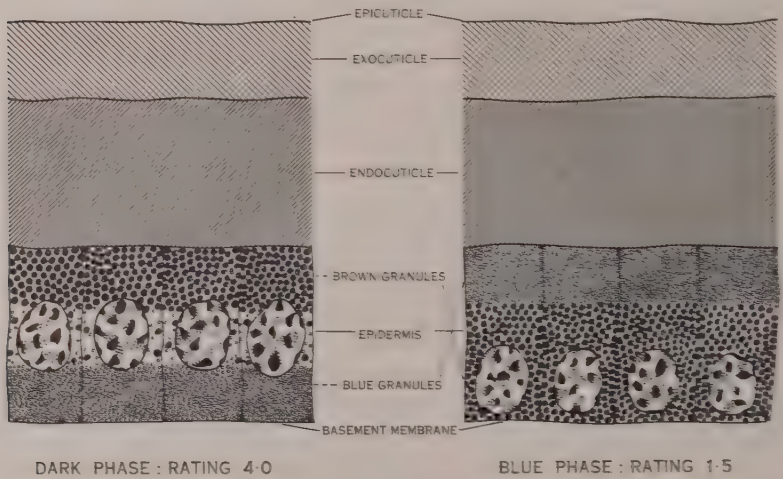

Fig. 2.—Transverse section of integument of the pronotal disk of a *K. tristis* male, showing the appearance and distribution, in the dark and blue phases, of the blue and brown granules and the nuclei. (Diagrammatic.)

The structure of the integument of the male is illustrated semi-diagrammatically in Figure 2. On the pronotal disk the transparent cuticle is about 35 μ thick. The exocuticle (10 μ thick) and the outer two-thirds of the endocuticle stained with fuchsin in Mallory's triple stain, whereas the inner third of the endocuticle stained blue. The epidermis ("hypodermis" of many authors) forms a simple epithelium of columnar cells, two to three times as deep as their maximal breadth, with which are associated occasional glandular and sensory cells and trichogens. The prominent ellipsoidal nuclei are approximately 10 μ in greatest diameter and contain coarsely granular chromatin and one or two nucleoli. The pronotum is underfolded to a considerable depth in its posterior part, and the cuticle of the underfolded integument is connected with that of the outer integument by means of narrow longitudinal bars of endocuticle. In the underfolded integument the cuticle is about half the thickness of that on

the disk; the epidermis is of the pavement type, about quarter to half the height of that on the disk, with most of the cells several times as broad as deep.

The integument of other portions of the body sectioned was similar to that of the pronotal disk, except that the epidermal cells were usually cuboidal in shape. The integument of the head was not studied. It is probable that that of the dorsal surface would resemble the pronotal disk.

Unstained sections of the pronotum of blue males showed, under dark-field illumination, a dense layer of pale blue granules in the distal portion of the epidermal cells. Beneath these was a dense layer of brown granules reaching to the proximal end. In dark males the blue granules were concentrated towards the proximal end of the cells and the brown ones towards the distal end (Fig. 2). Thus it appears that the colour change is due to the predominance, at the upper surface of the epidermis, of either blue or brown granules, which move in opposite directions within each cell as the change proceeds.

The brown granules were spherical, of somewhat variable size averaging about 1 μ, and dark brown in colour. The "blue" granules were smaller and more densely crowded than the brown and about 0·3 μ in diameter, i.e. at about the limit of optical resolution. They were brilliantly refractive, the apparently very pale blue colour being possibly a diffraction effect. In view of their small size, no significance should be attached to their apparently spherical form: they may well be crystalline. In sections stained with Mallory's triple stain and examined by transmitted light, the brown granules had much the same appearance as in unstained preparations. The blue granules had lost their brilliance and colour, and their individuality was by no means as evident: they appeared as a pale zone or a misty suffusion within the cell. When spreads of the isolated epidermis were treated with strong acid or alkali the brown granules turned red.

Brown and blue granules could be seen in the epidermis of the other body regions studied, although in places one or the other, or both, were absent. They were even present in the underfolded integument of the pronotum, where the brown granules were concentrated along the distal (in this case the *ventral*) face of the cells in the dark phase and along the proximal face in the blue phase, just as on the disk.

The distribution of the two types of granule in relation to colour rating of the male was studied on the basis of the pronotal disk—both because the rating is based on this area and because the columnar shape of the cells results in a more evident separation of zones. At a rating of 1·5 (Fig. 2; Plate 2, Fig. 1) the brown granules are confined to the proximal two-thirds to three-quarters of the cells, resting solidly on the basement membrane at the proximal end and sharply delimited at the distal end. The blue granules occupy the distal quarter to third, resting immediately upon the brown zone, but apparently not overlapping it. The nuclei rest on the basement membrane. At a rating of 2·0 (Plate 2, Figs. 2 and 4) there is a slight tendency for the brown granules to lift from the basement membrane. Otherwise the cytological picture is that of the 1·5 rating, except that there is perhaps a slight overlap between the two zones. At 2·5 (Plate 2, Figs. 3 and 5) a further lifting of the brown granules above the basement membrane has occurred, so that only a few scattered

granules remain in the proximal quarter of the cells, where the nuclei, slightly overlapped by the brown zone at their distal ends, are still located. The brown zone occupies roughly the central half of the cell, the blue zone above it having narrowed through the distal advance of the brown granules. The blue granules are densest in the distal blue zone, but they are also distributed at an almost uniform lower density throughout the cells, reaching even to the proximal end. At 3·0 the brown granules extend to the distal end of the cells, the brown zone occupying the distal third to half. The proximal margin of the brown zone has lifted further, and the nuclei have also begun to lift from the basement membrane, maintaining contact with the brown zone at their distal extremities. The blue granules are still distributed throughout the cells, but their density at the distal end has diminished and a distinct concentration is present at the proximal end. At 3·5 (Plate 2, Fig. 6) the picture is much the same as at 3·0. The brown zone is perhaps a little narrower, and the nuclei, which are tending to lose contact with the granules, a little more raised. In the darkest phase, represented by a rating of 4·0 (Fig. 2; Plate 2, Figs. 7, 8, and 9), the brown pigment is more densely concentrated in a slightly narrower band, the nuclei are almost in the centre of the cells in contact with the brown zone, and the blue granules are densely concentrated in the proximal quarter, practically none being visible elsewhere in the cell. The region of the cells containing the nuclei is thus practically free from both types of granule (Plate 2, Fig. 7), while the positions of the blue and brown zones are completely inverted in comparison with the blue phase (Fig. 2).

The above descriptions reflect the general cytological picture on the pronotal disk at each colour rating. However, as may be seen from Plate 2, there is some variation in the response of individual cells. This variation is somewhat wider when one compares more remote areas of the disk. In particular, there is a regular tendency, in the blue phase, for the superficial blue zone to be narrowest beneath the slight integumental depressions and broadest beneath the elevations, especially beneath the mid-dorsal carina. Conversely (Plate 2, Fig. 9), the superficial dark zone of the dark phase is broadest in the depressions and narrowest in the elevations.

It may be seen that up to the rating 2·0 the cytological picture is essentially that of the blue phase. Variation in rating below 2·0 may be largely due to genetic differences between individual insects (cf. previous section). The rating 2·5 is cytologically a markedly intermediate stage. The brown granules are fully half way to the position characteristic of the maximally dark insect, while the blue granules are already distributed throughout the cell, although they still show a distal concentration. By 3·0 the brown granules have occupied essentially their position in the dark insect, while the blue granules are already concentrating at the proximal end. The remaining changes consist only of a slight further concentration of the brown granules and the withdrawal of all the blue granules into a dense proximal zone. Thus the greater part of the cytological difference is already evident between ratings 2·0 and 3·0. The brown granules complete their change of position within a narrower rating range than the blue ones; they also seem to move more as a band, advance of the distal

"front" being accompanied by lifting of the proximal margin. The blue granules seem to disperse from the distal concentration and become absorbed in a proximal concentration, both concentrations existing simultaneously at about 3·0.

It must be remembered, however, that the preparations studied were derived from insects fully accommodated to the appropriate temperatures, and that in the actual process of accommodation the movements might take a different course. If the temperature changed so gradually from a high to a low level (or vice versa) that the changing colour was always virtually in equilibrium with the temperature of the moment, one could expect that the cytological picture corresponding to each colour rating would be as described above; but an insect transferred suddenly from a high to a low temperature might pass through a colour rating of, say, 3·0, in which the distribution of the granules might be different. Everything would depend upon the relative rates of movement of the two types of granules at the given temperature. The cytological observations here reported can make no contribution to this question of rate of movement.

Sections of the integument of pale and dark females, of both the brown and green forms, revealed essentially the same cytological picture as in the male. That is, in the pale phase the superficial region of the cells is occupied by very pale blue, highly refractive granules, and the lower portion by larger dark brown granules, while in the dark phase the position of the two types of granule is reversed. The only readily discernible difference between the sexes is in the pale phase, where in the female the blue zone occupies only about one-sixth of the height of the cells and the brown zone thins out very considerably in about the proximal quarter to third of the cells, being dense only in a central region.

Thus, while it can hardly be doubted that in both sexes paleness or darkness depends upon the extent to which the pale refractive granules or the dark brown granules are predominant in the surface layers of the epidermis, the difference between the striking blue of the pale male and the green or brown of the pale female remains unexplained. The only pointer provided by the cytological observations is the greater shallowness of the blue zone in the pale female and the probably smaller number of "blue" granules in that sex. No histological difference could be detected between green and brown females in either the pale or dark condition. All these differences are probably due to differences in the occurrence and distribution of pigments that had been leached out of the preparations by alcohol or xylol (see Section V).

No pigment migration could be detected in the eyes of *K. tristis*.

IV. Observations and Experiments on Other Acrididae

(*a*) *Species of* Kosciuscola

Reference has already been made to the undescribed "*Kosciuscola* sp. 2", which occurs along with *K. tristis* on the higher levels of the Kosciusko Massif. A third species, which we may call "*Kosciuscola* sp. 3", occurs in the same region from about 4000 ft to about 5500 ft. Specimens of both sexes of these species were subjected to a constant temperature of 4°C overnight and to 35°C for some hours. In no case was there any discernible difference in the colour of the

dorsal or lateral surfaces of the insects that could be ascribed to the difference in temperature. On the other hand, the face and ventral surface showed a colour response analogous to that of the same areas in the female of *K. tristis*.

The colour of these parts in *K.* sp. 2 was in general as follows: At 4°C the face was darker, the portion above the clypeus being brown in the male. Mesometasternum brown to very dark brown, pale along the sutures and posterior margin. Abdomen brownish to dark brown, the posterior margins of the sternites pale. At 35°C the face was paler, and often greener above the clypeus. Mesometasternum pale bluish green to pale greenish buff, darker along the sutures. Abdomen pale buff or pale gray, sometimes with a greenish tinge, anterior margins of the sternites dark.

K. sp. 3 showed the following differences: At 4°C, face darker, brown to very dark brown above the clypeus, except in some females where this area was green. Mesometasternum brown to very dark brown, except along the sutures and posterior margin. At 35°C, both these regions were very pale gray or grayish white to pale buff. No clear differences on the abdomen.

Sections were cut of the pronotal disk and the frontal region of the head of both species. Both blue and brown granules, apparently similar to those of *K. tristis*, were present in most of the epidermal cells of both sexes in both species.

In sections of the pronotal disk the blue granules occupied usually about the distal fifth of the cell at both temperatures. The brown granules were distributed through the remainder of the cell, but at 35°C were usually concentrated most densely in a central region. In sections of the frons the picture was by no means so clear. At 4°C the granule distribution differed widely in adjacent cells and groups of cells. Cells with the brown granules in the distal portion predominated; in these, blue granules were sometimes absent and sometimes concentrated proximally. In some cells the blue granules occupied the distal position. Only one insect kept at 35°C was sectioned. In this there appeared to be no typical blue granules. The brown granules occupied consistently the proximal portion of the cell, the distal portion being non-refractive.

From the above observations we may conclude that the coloration of the dorsal and lateral surfaces of *Kosciuscola* spp. 2 and 3 corresponds to the pale condition in *K. tristis*, and that the colour change in the latter species is essentially a mechanism for reversible darkening. Further, whether we regard *K.* spp. 2 and 3 as in the process of acquiring the complete colour response shown by *K. tristis* or as having lost the capacity to respond on the dorsal and lateral surfaces, it appears that the capacity to respond on the face and under surface is of the first importance. This is in accordance with the observation (Section III (*a*) (i)) that in *K. tristis* it is the face and under surface that change colour most rapidly when the temperature is changed; however, it complicates the interpretation of the ecological significance of the colour change.

(*b*) *Species Belonging to Other Genera*

Material of the following species* was collected at Canberra in order to determine whether any of them showed a colour response analogous to that of

the genus *Kosciuscola*: *Praxibulus laminatus* (Stål), *Phaulacridium vittatum* (Sjöst.), *Peakesia fuscomaculata* Sjöst., *Macrotona australis* (Walk.), *Acrida conica* (Fabr.), *Cryptobothrus chrysophorus* Rehn, *Aiolopus tamulus* (Fabr.), *Austroicetes pusilla* (Walk.), *Gastrimargus musicus* (Fabr.), and *Oedaleus australis* Sauss. Material of *Locusta migratoria* L.* was obtained from cage-bred stocks reared from adults taken at Sydney, N.S.W.

Individuals of these species were paired off according to general similarity of colour and pattern and retained overnight at about 15·6°C. The main features of the coloration of each specimen were noted, paying special attention to differences between the members of a pair. One member of each pair was then placed at 4°C and the other at 35°C. After about 4 hr they were compared again and the differences within the pairs noted. The insects previously at 4°C were then placed at 35°C and vice versa, and a second comparison of the members of pairs was made after a further 4 hr. The only species to show any trace of colour change was *Oedaleus australis*, of which a single pair was used. The insect kept at the lower temperature had the darker face at both comparisons.

Five pairs of *O. australis* males and three pairs of females were then left overnight at 15·6°C and the following morning one member of each pair was placed at 4°C and one at 35°C. After 2½ hr a comparison of the pair members, which had been matched initially as described above, showed that, in four of the five male pairs, and in all three female pairs, the member at the lower temperature was somewhat darker than that at the higher one. The face was the area most consistently affected, but the sternum, pronotum, and gena were often also involved; the males showed the difference more clearly than the females. When the specimens at 4°C and 35°C were then interchanged and compared 3 hr later, the expected reversal of depth of colour did not occur. In only one pair was the insect at 4°C the darker; in general the insect at 35°C was slightly the darker or there was no noticeable difference. A comparison the next morning gave the same result.

The existence of a colour response in *Oedaleus australis* analogous to that of *Kosciuscola tristis* thus remains unproven, although the probability is that it does exist but that the conditions for its optimal operation have not yet been found. It is possible, for example, that a temperature as low as 4°C inhibits change in either direction, so that insects transferred from 4°C to 35°C show little difference from those whose colour at 35°C has been "fixed" by transfer to 4°C. It should be noted that *Praxibulus laminatus*, which showed no colour response, is closely related to *Kosciuscola*, while *Oedaleus australis* belongs to a different subfamily of the Acrididae.

V. Discussion

Among the Acridoidea, physiological colour change has hitherto been unknown. Much work has been done on morphological colour change in this

* Identification by K. H. L. Key. Some names likely to be altered as a result of current work by J. A. G. Rehn.

group (cf. Faure 1932; Hertz and Imms 1937; Ergene 1950, 1952*a*, 1952*b*; Joly 1951, 1952; Burtt 1951). In every instance the colour change has required several days, and often the intervention of a moult, for completion, and appears to depend upon the metabolic production or destruction of pigment rather than its migration within the cell. Although Burtt (1951) states that the blackening of *Aulacobothrus wernerianus* (Karny) on burnt grass in the sun is "sometimes noticeable after as little as two days," and Kopenec (1949) found that the physiological response of *Corethra plumicornis* larvae on a white background took as long as 1-3 days, yet in *Kosciuscola tristis* and most other animals showing a physiological response the colour change is clearly evident within ½-1 hr and practically complete in less than a day. Certainly no evidence pointing to a physiological response has hitherto been adduced for any of the Acridoidea.

The closest parallel to the colour change in *K. tristis*, both in its cytological and physiological (see Key and Day 1954) aspects, is provided by the phasmid *Carausius morosus* Brunn. & Redt. (Giersberg 1928 and others). However, the latter species shows several noteworthy cytological differences from *K. tristis*. According to Giersberg, darkening is brought about by the simultaneous movement of two types of granules. In the pale phase, dark brown granules are concentrated in a compact mound on the basement membrane of each epidermal cell, while a smaller clump of smaller, orange-red granules is situated near the distal margin of the centrally located nucleus. Neither of these concentrations forms a distinct layer extending at equal depth to the lateral margins of the cell, as do the two pigment layers in *K. tristis*, and much smaller numbers of granules seem to be present. In the dark phase the brown granules have migrated distally to form a shallower and more laterally extended layer, which, however, does not appear to be continuous along the epidermis as in the dark phase of *K. tristis*. The orange-red granules do not change their position along the proximo-distal axis of the cell, but spread laterally to form a thin and apparently almost continuous layer instead of a series of clumps. There is nothing corresponding to the blue granules of *K. tristis*, and no movement of granules in opposite directions within the cell. Janda (1935) gives figures showing a less clumped distribution of the brown granules in the pale phase, and a nearly continuous layer of them in the dark phase. His figures also show quite clearly that the nuclei are situated on the whole more distally in the dark phase than the pale.

In addition to the two pigments mentioned, Giersberg reports two others in *C. morosus*: a green and a yellow one, both occupying the distal half of the epidermal cells and not migrating. Both of these pigments, as well as the orange-red one, are soluble in alcohol, while the yellow and orange-red ones are also soluble in fat solvents. Thus, if analogues of any of the three are present in *K. tristis*, they would be removed by the conventional histological procedure adopted in the present investigation (Section III (*b*)). This has at least three consequences in connection with the interpretation of the cytological observations on *K. tristis*. In the first place, we are not in a position to state that a lateral migration of pigment, as found in *C. morosus*, does not

occur in *Kosciuscola*; secondly, we have to consider the possibility that the "blue" granules of *Kosciuscola* may have been leached of pigment by the histological procedure; and, thirdly, the possibility of the differential occurrence of alcohol-soluble green, yellow, and perhaps blue pigments, in either granular or diffused form, may afford an explanation of the colour differences between males, green females, and brown females, all in the pale condition, for which no adequate reason seemed to be provided by the distribution of the observable granules (Section III (*b*)).

It has been shown in Section II that the behaviour regime of *K. tristis* in its natural habitat is such, within the range of temperature experienced during the authors' field observations, that the insect occupied, almost throughout the 24 hours, those stations where it would be expected to have the highest internal temperature. It was also pointed out that the capacity of an alpine grasshopper to utilize solar radiation as a means of raising its internal temperature must be one of the most significant factors affecting its survival. On the assumption that, at least under some conditions of heat exchange, an insect in the dark phase is able to maintain a higher internal temperature in the sunshine than one in the blue or pale phase, one would be disposed to suggest an ecological significance for the capacity to assume a dark matt colour at those times (early morning and evening; cold, windy, and slightly overcast periods) when even a small difference in internal temperature could be important in metabolism, and hence in rate of development and the ability to reproduce within a short growing season.

This hypothesis of a thermoregulatory function for physiological colour change in poikilotherms is by no means new. It was put forward by Weber as long ago as 1881 and examined in detail by Bauer (1914). More recently it has been invoked by Brown and Sandeen (1948). The hypothesis receives perhaps its strongest support from the responses of certain lizards which, having spent the night in the pale phase (as a response to darkness at moderate temperatures) become dark during the early morning and then pale once more during the heat of the day (as a reaction to high temperature). It is contended that by these changes of colour the absorption of radiation through the skin is so regulated as to maintain the internal temperature of the animal at the most favourable level possible under the prevailing environmental conditions (Bauer 1914).

Until quite recently, the assumption that the dark form of a polymorphic grasshopper had a higher internal temperature in the sun than the pale form of the same species was considered to be well established. Thus Buxton (1924) showed that a dark form of "*Calliptamus coelesyriensis*" (*Metromerus coelesyriensis* (Giglio-Tos)) became 4-5°C warmer than the pale form. Bodenheimer (1934), working on the same species, found a difference of 2-3°C. Hill and Taylor (1933) found a difference of 3°C between dark gregarioid and pale solitarioid nymphs of "locusts" (according to Uvarov (1948) these were *Locusta migratoria* L.). Finally, Strel'nikov (1932, 1936) obtained, in various comparisons, differences of 2·9-6·6°C between gregarioid (dark) and solitarioid

(pale) nymphs of *L. migratoria.* (See also Gunn (1942) and Uvarov (1948).) Parry (1951) states that there may be a variation of up to 50 per cent. in the total radiation load of terrestrial arthropods due to the colour of the animal alone.

Pepper and Hastings (1952), however, using a non-reflecting light cone designed to prevent as far as possible all factors other than direct solar radiation from influencing the internal temperature of the grasshoppers they insolated, found no significant difference between the internal temperatures of "black" and "yellow" forms of *Melanoplus differentialis* (Thomas) after temperature equilibrium had been attained. Nevertheless, the black forms reached equilibrium more rapidly. Pepper and Hastings do not attempt to reconcile their findings with those of the earlier authors quoted, but it is possible that the very refinement of their technique excluded factors of crucial importance in bringing about a temperature difference between dark and pale forms under more natural conditions of exposure to insolation. In particular, the factor of convection was excluded. It can be shown that, the more factors other than radiation predominate as causes of heat loss (and of these convection is the chief), the greater will be the tendency to a temperature difference between dark and pale insolated bodies. Thus it would seem justifiable to accept the evidence of the field workers and to adhere to the assumption that at times *K. tristis* in the dark phase would reach, in the sun, a higher internal temperature than the pale phase under the same field conditions.*

The suggested ecological significance, for an alpine grasshopper, of being able to turn dark, would seem to attach equally to a grasshopper that was permanently (genotypically) dark in colour. Indeed, a black tettigoniid, *Acripeza reticulata* Guér. occurs on the Kosciusko Massif, and a black acridid on the highest levels in Tasmania ("Genus and sp. n. 2" of Key (1952)). In both these groups of Orthoptera an obligatory black colour is most unusual, only one such Australian acridid being known to us apart from the Tasmanian species. (Cf. the controversial paper by Kalmus (1941), especially his "rules" 2 and 11. However, Kalmus's theories were developed for cuticular colour.) On the other hand, the distinctive feature of the colour change in *K. tristis* is that it not only allows the insect to be dark at low internal temperatures, but also produces a paling at high internal temperatures. To explain the evolution of the mechanism along thermoregulatory lines we have therefore to postulate an advantage in avoiding overheating under the conditions when the insect is pale. Bauer (1914) has pointed out that poikilotherms can regulate their internal temperatures within very wide limits by taking up appropriate stations within the environmental complex, and that this ability makes it unnecessary to regard

* Internal temperature measurements on *K. tristis* made by us at Canberra failed to show a significant difference between the two phases. These measurements are open to the same objection as those of Pepper and Hastings, so far as their applicability to field conditions is concerned, in that we have no assurance that the general conditions of heat exchange under which they were made would have permitted a temperature difference to be registered.

colour change or any other physiological regulatory mechanism as essential to these animals when living in an environment subject to extreme fluctuations of temperature. This should, indeed, be obvious from the fact that most insects, for example, do not show colour change and have but slight capacity to regulate their temperature by any physiological means.

It is characteristic of the Acrididae in particular that, just as they seek out sunlit places for "basking" when their internal temperature is below optimum, so they seek shade, or expose themselves to the wind, when they are becoming over-heated; also, presumably, they may succeed in so placing themselves, within a pattern of sun and shadow, that the appropriate fraction of their surface is insolated to maintain the optimal internal temperature. The radiation load can also be altered by suitable orientation of the long axis of the body to the sun's rays. Volkonsky (1939) has shown that an internal temperature difference of 10°C can be produced as between the positions of "menakinesis" and "telakinesis," and Parry (1951) calculated that the mean radiation load of a body with markedly unequal faces may be varied by a factor of 2 according to its orientation.

However, these usual methods of regulating internal temperature involve a good deal of movement in search of appropriate conditions, and then restriction to the areas possessing them; or else they involve the maintenance of a constant orientation to the sun's rays, which in any case is by no means always sufficient to maintain the internal temperature near the optimum. Neither the movement nor the restriction of station or orientation may be favourable from the point of view of other activities in which a grasshopper may need to indulge, e.g. feeding, oviposition, etc. A black grasshopper might be expected to be particularly burdened by the forced movements and the restriction of location and orientation; for it is more susceptible to over-heating in the sun and to over-cooling in the shade (by re-radiation to surrounding cooler objects) than a pale one. On the other hand, a black grasshopper with the power of becoming progressively paler as its internal temperature rises, and thus of restricting further rise, would have greater freedom to go about its business in the sunshine without becoming either over-heated or over-cooled. It could reap the advantages of blackness without being saddled with its disadvantages. The benefit of such a mechanism would seem to be greatest in an alpine environment, where solar radiation is intense but the shade temperature low, and where it is important that there should be no restriction upon any activities connected with rapid development and reproduction. It may be significant that the black Tasmanian acridid already referred to is confined to slopes of coarse talus, where it utilizes the crevices between the rocks for escape from attackers, and, presumably, for protection from both heat and cold (unpublished observations by K. H. L. Key).

If there is any truth in these speculations, it may not be merely coincidental that Slifer (1953*a*, 1953*b*) has found that *K. tristis* alone, among 122 species of grasshoppers she examined, lacks the characteristic thermoreceptors to which

she assigns a role (Slifer 1951) in orientation to sources of radiation.* For the more an insect can afford to disregard radiation intensity, the less will it need such organs. That *K. tristis* still possesses the power of orientation is shown by its adoption of the menakinetic attitude in the early morning (Section II). The eyes are presumably adequate receptors for this purpose. It is not known whether it ever adopts the telakinetic attitude.

The occurrence of the colour change on the ventral surface of *K. tristis* (Section III (*a*)), the speed with which the change is completed there, and the restriction of the response to the ventral surface and face in *Kosciuscola* spp. 2 and 3 are not easy to reconcile with the scheme set out above. It is true that soil and rocks become heated by solar radiation and can impart some of that heat, both by radiation and conduction, to animals resting upon them. Swan (1952), whose observations on Mt. Orizaba, Mexico, are of particular interest to this study because the mean annual temperature at the altitude at which he worked is only 1-2°C different from that of the Mt. Kosciusko area, found that the surface of rocks could be raised to 40°C in the sunshine, when the air temperature was about 10°C. But it is most unlikely that the surface of either rocks or soil could rise in temperature as rapidly as a black grasshopper, because of their conductivity and, in the case of soil, its frequently high water content. In the early morning, therefore, when the insects are dark, they would lose rather than gain heat through the ventral surface of the body by sitting on soil or rocks. During the greater part of a sunny day, their pale ventral surfaces would seem adapted to reflect rather than absorb heat from the substratum, and in the evening they seem to retreat into shrubs before becoming dark enough to absorb much radiation from below. In any case, Parry (1951) has concluded that radiation from heated ground can make only a small contribution to the higher temperature of an insolated black body very close to the ground in comparison with one in "free air," the difference being due mainly to the higher air temperature and reduced wind speed near the ground.

It cannot be said that these speculations offer a convincing explanation of the ecological role of the colour change in *K. tristis*. They do, however, indicate a variety of possibilities which future workers should be able to test by appropriate observations in the field. It seems most unlikely that the common interpretation of colour change, in terms of the protection afforded an animal by virtue of its colour or pattern in relation to its background (cf. Cott 1940), will have any application to *K. tristis*. At the same time, we should bear in mind Bauer's (1914) caution that colour change may fulfil more than one function simultaneously.

* In a personal communication Dr. Slifer has informed us that *K.* sp. 3, which has a rudimentary colour change (Section IV (*a*)), has only two pairs of thermoreceptors in both sexes; this is the smallest number in any acridid examined by her, except for *K. tristis*. We thus have a gradation from (marked colour response; no thermoreceptors) through (weak colour response; two pairs thermoreceptors) to (no colour response; multiple thermoreceptors). *Oedaleus australis* has a normal complement of thermoreceptors (Slifer 1953*a*, 1953*b*).

VI. Acknowledgments

The authors are indebted to Mr. L. A. Marshall, Division of Entomology, C.S.I.R.O., for preparing the illustrations and particularly for the splendid paintings reproduced on Plate 1; to Mr. D. Wilson, Division of Entomology, C.S.I.R.O., for the photomicrography; to Mr. B. B. Given, Department of Scientific and Industrial Research, New Zealand, for procuring the material on which the preliminary observations and experiments were carried out; to Mrs. M. J. Bennetts for assistance with the histological preparations; and to Mr. G. A. McIntyre, Section of Mathematical Statistics, C.S.I.R.O., for assistance in the evaluation of quantitative data.

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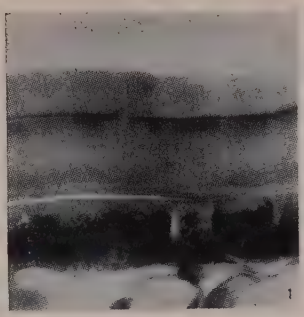
COLOUR RESPONSE TO TEMPERATURE IN *KOSCIUSCOLA*

Fig. 2

Fig. 1

COLOUR RESPONSE TO TEMPERATURE IN *KOSCIUSCOLA*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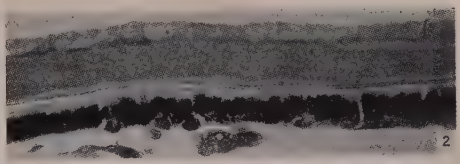
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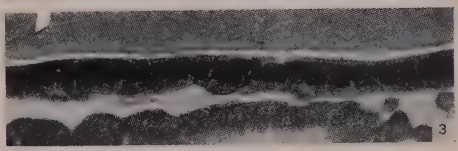
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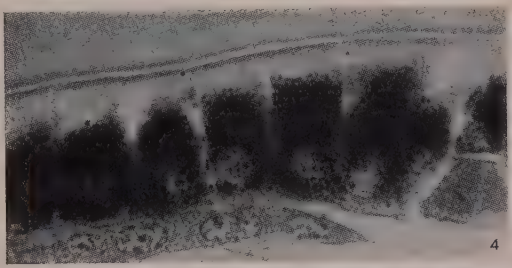
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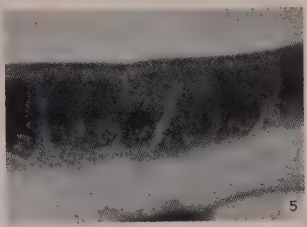
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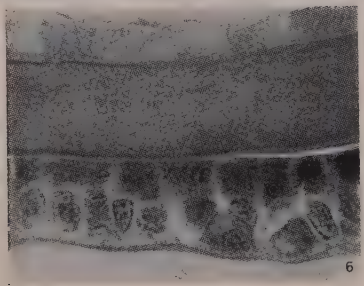
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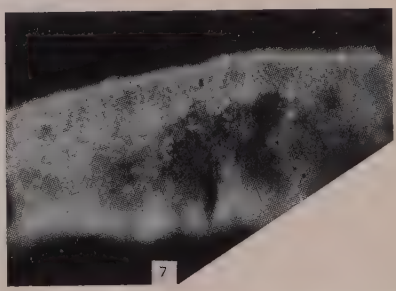
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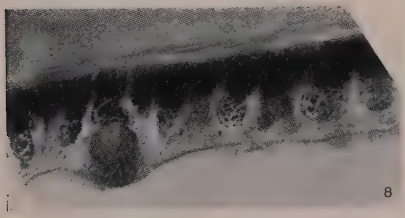
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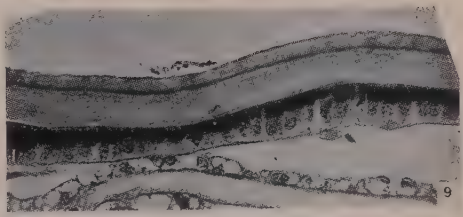
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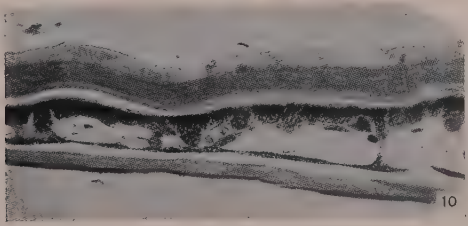
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Explanation of Plates 1 and 2

Plate 1

Kosciuscola tristis, male.

Fig. 1.—Dark phase, colour rating 4·0.
Fig. 2.—Blue phase, colour rating 1·5. × *c.* 7.

Plate 2

Photomicrographs of transverse sections of the pronotal integument of *Kosciuscola tristis*, showing the distribution of the granules and nuclei of the epidermal cells corresponding to different colour ratings. Viewed by transmitted light except in Figure 7.

Fig. 1.— ♂ , rating 1·5. Fig. 2.— ♂ , rating 2·0. Fig. 3.— ♂ , rating 2·5. Fig. 4.— ♂ , rating 2·0. Fig. 5.— ♂ , rating 2·5. Fig. 6.— ♂ , rating 3·5. Fig. 7.— ♂ , rating 4·0; dark-field illumination. Fig. 8.— ♂ , rating 4·0. Fig. 9.— ♂ , rating 4·0. Fig. 10.— ♀ , brown form, pale phase.

THE PHYSIOLOGICAL MECHANISM OF COLOUR CHANGE IN THE GRASSHOPPER *KOSCIUSCOLA TRISTIS* SJÖST. (ORTHOPTERA: ACRIDIDAE)

By K. H. L. Key* and M. F. Day*

[*Manuscript received April* 22, 1954]

Contents

Summary

Laboratory experiments are described in which the colour response of male *Kosciuscola tristis* to temperature was analysed. When the temperature was changed, the colour, assessed quantitatively by means of a colour chart, varied according to time-curves approaching asymptotically a new equilibrium value. The rate of change during the early stages of accommodation is correlated with the difference between the initial and final temperatures, while the mean rate of change over the first 80 per cent. of the change is related rather to the final temperature. Other things being equal, the change from dark to blue is more rapid than the reverse change. The equilibrium colour, attained after about 18 hr at constant temperature, is related to temperature by a sigmoid curve flattening out at about 10 and 27°C.

Experiments in which light intensity, background colour (black or white), relative humidity, and degree of crowding were varied at constant temperature showed that these factors are without influence on the colour of the insect.

Experiments in which different parts of the intact insect, isolated halves of insects, and pieces of integument *in vitro* were subjected to different temperatures showed that nervous or hormonal coordination is not involved in the response, the epidermal cells acting as independent effectors.

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I. Introduction

In an earlier paper the authors described (Key and Day 1954) a temperature-controlled physiological colour response in the alpine grasshopper *Kosciuscola tristis* Sjöst., paying particular attention to the relation of the response to the ecology of the insect. The present paper deals with laboratory experiments undertaken with a view to analysing the conditioning of the response and its physiological mechanism.

The experiments were carried out during March and April 1952, using material obtained from the Kosciusko Massif, New South Wales. This comprised several hundred males of *K. tristis*, which were kept in wood-gauze-glass cages at room temperatures of about 20-24°C. Plentiful food was supplied in the form of tussocks of *Poa australis* and bundles of other species of grass, the latter being renewed daily. Dead grasshoppers were removed daily and the cages cleaned out from time to time. The grasshoppers fed readily upon the grasses and appeared to be in excellent health for at least the two weeks during which the main experiments were completed. A small percentage were parasitized by the larvae of a tachinid fly, *Myothyria armata* Mall.,* a small number of the puparia of which were found on most days on the floor of the cages.

II. Reliability of the Rating Method

In all the experiments to be described, the colour of the insects was rated visually by means of the colour chart described by Key and Day (1954). Methods of visual rating of biological phenomena are quite widely used in ecological and agricultural research. Although they are not so familiar to laboratory workers, they have already been used extensively by students of colour change (cf. Brown 1946). The results obtained by such methods can be manipulated in the same way as physical measurements, even if there is no assurance that the rating scale corresponds to an arithmetical progression of any theoretically measurable quantity. In this connection the critical comments of Parker (1943) are relevant only where such a correspondence is unjustifiably assumed. However, the success of the method depends upon the reproducibility of its ratings, and this in turn demands a careful standardization of the rating procedure, with the elimination, as far as possible, of the influence of subjective bias.

The great majority of the ratings were made by the two authors in consultation. Agreement was readily reached, so that it is unlikely that any large personal factor obtruded on the occasions when ratings had to be made by one author only. The insect to be rated was confined in a thin glass specimen tube 1⅞ in. long and ⅝ in. dia., in which its freedom of movement was greatly restricted. The tube was held so that the insect faced the observer, and the pronotal disk was rated with the aid of a light above the observer.

The reproducibility of the ratings was investigated, in principle, by repeatedly rating the same insects, which had previously been fully equilibrated and were maintained at near-constant temperature. An analysis of the data

* Identification kindly provided by Dr. S. J. Paramonov, Division of Entomology, C.S.I.R.O.: specimens deposited in the Division of Entomology Museum, Canberra.

obtained is presented in Appendix I, which also gives information on the variance between individual insects based on a single rating each. The results of the analysis may be summed up as follows. Over the greater part of the rating range the subjective error of rating on any one occasion has a variance estimated as 0·0322 and in addition there is possibly a small systematic error of rating, shifting from occasion to occasion, the variance of which has been estimated very approximately as 0·0082. Between insects under the same temperature conditions there is an inherent variance of the order of 0·10. All these variances fall sharply to zero at a mean rating of 4·0. The variance attaching to the mean of single ratings of n insects is then $0{\cdot}13/n$ for comparisons within occasions and $0{\cdot}13/n + 0{\cdot}008$ for comparisons between occasions involving different groups of insects. For treatment comparisons of changes within insects due to temperature, the appropriate error variance is given by the variance of such changes.

III. Experiments on Conditioning Factors

(*a*) *Possible Influence of Light, Background, Humidity, and Crowding*

To determine whether factors other than temperature had any influence on the colour change, the factors light intensity, background colour, relative humidity, and degree of crowding were tested. The experimental design necessarily varied somewhat according to the factor whose possible influence was being investigated, but all experiments were run concurrently on the same bench, using *K. tristis* males from the stock cages, which were kept in the room where the experiments were done. Temperature conditions within the stock cages probably varied somewhat from point to point and also differed somewhat from those on the experimental bench, so that rating changes between the initial and final ratings of the experiments could reflect a real adaptation to a slight temperature change upon transfer from the stock cages, as well as to the slight temperature changes on the bench during the course of the experiments.

Each experiment employed 40 insects, a random half of which were exposed to one extreme level of the factor being investigated and the other half to a widely different level. The insects were rated immediately before the start of each experiment, and again after 5 hr, when the experiments were terminated. Within each experiment, the influence of the two levels of the factor studied was compared on the basis of the change between the initial and final ratings. Care was taken to equalize all factors, other than the one being investigated, as between the two groups of insects.

The detailed arrangement of the experiments was as follows:

(i) *Light.*—The levels of light intensity used were complete darkness and diffused daylight received through a large window immediately behind the bench. Four measuring cylinders, each about 1⅞ in. dia. and 12-15½ in. long, were completely covered with a heavy black paper to exclude light, and then with white paper (except at the extreme ends) to reflect light and radiant heat. A further four similar cylinders were left uncovered, except at the open end, which was covered with white paper. The cylinders were arranged in two ranks

symmetrically placed with relation to the window, the darkened and undarkened cylinders being alternated within each rank and staggered as between ranks. A thermometer was placed inside each cylinder, as well as in the centre of the group of cylinders. The 40 insects were randomly divided into eight groups of five, each insect being contained in a 1⅞ by ⅜ in. numbered glass tube plugged with cotton wool. After rating, one group was placed in each cylinder, the tubes being disposed as far as possible end to end on the window side of the thermometer stem, when they extended for some two-thirds of the length of the cylinder from its base. In this position the insects in the undarkened cylinders received light mainly at right angles to the long axis of their bodies. The paper covers at the open ends of all cylinders were then folded over and sealed.

(ii) *Background.*—Four circular tin trays, 3 in. dia. and about ¾ in. deep, were lined on the bottom and sides with black paper, and a similar four with white paper. The trays were covered with 1/16-in.-mesh wire gauze and arranged in two blocks of four symmetrically placed with relation to a large window, the positions of the black and white trays in the one block being interchanged in the other. Two further trays, one lined with black paper and the other with white, were provided with slits in the sides to allow of thermometers being inserted with their bulbs resting on the bottom of the trays. These were also placed symmetrically in relation to the window. The 40 insects were randomly divided into eight groups of five; after rating, one group was placed free in each tray. A thermometer was placed in the centre of the group of trays.

(iii) *Humidity.*—Two similar desiccators, 6 in. dia., one containing tap water and the other anhydrous $CaCl_2$, were placed symmetrically with relation to two large windows. A thermometer was placed between them. The 40 insects, each contained in a numbered 1⅞ by ⅜ in. glass tube closed by mosquito netting attached by a rubber band, were randomly divided into two equal groups and rated. One group of tubes was placed in each desiccator, the tubes being disposed more or less parallel to one another in two rows, with the netting-covered ends away from the light source. The positions of the desiccators were exchanged after 2, 4, and 4½ hr, so that each desiccator spent the same time in each position, and not more than 30 min in the final position before the final rating.

(iv) *Crowding.*—A block of 21 cylindrical wire-gauze cages 3¾ in. high and 3 in. dia., fitted with wire-gauze lids having a ¾-in. circular opening in the middle, was symmetrically disposed in front of a large window. Each cage was completely screened off from every other by cardboard sheets. This resulted in considerable variation in light intensity as between cages. After rating, 20 insects were placed free in a cage in the centre of the block, and one insect free in each of the remaining 20 cages; the openings in the lids were then plugged with cotton wool. A thermometer was placed near the centre of the group of cages.

None of the thermometers used in these experiments recorded a change of temperature, between the initial and final ratings, of more than 1·6°C. In the experiment with light, the mean temperature given by the thermometers in the four darkened cylinders at the time of the final rating was 23·85°C and the

mean for the four undarkened cylinders 23·92°C. In the experiment with background, the thermometer in the black tray read 23·1°C at the time of the final rating and that in the white tray 22·9°C. Thus there was practically no temperature difference between the groups of insects being compared, and only a very small change over the period between the initial and final colour ratings.

The results of the experiments are set out in Table 1. In no case did the different levels of the factors studied have any significant effect on the colour rating and there is no suggestion that they might have such an effect given longer exposure.

(b) Colour Accommodation upon Transfer from One Temperature to Another

The change of colour consequent upon a given change of temperature was investigated by transferring groups of 10 *K. tristis* males, which had previously been equilibrated for 16-20 hr at near-constant temperatures of about 18 and

TABLE 1

MEAN COLOUR RATINGS AND MEAN ENVIRONMENTAL TEMPERATURES FOR GROUPS OF 20 *K. TRISTIS* MALES AT THE COMMENCEMENT AND CONCLUSION OF 5 HR EXPOSURE TO CONTRASTED LEVELS OF LIGHT INTENSITY, BACKGROUND COLOUR, RELATIVE HUMIDITY, AND DEGREE OF CROWDING

Factor	Treatment	Mean Rating		Mean Temperature (°C)		Analysis of Variance* (mean squares)	
		Initial	Final	Initial	Final	Treatments	Sampling Error
Light intensity	Light	2·33	2·35	23·0	23·92	0·0125	0·0422
	Dark	2·38	2·25		23·85		
Background colour	White	2·60	2·50	24·3	22·9	0·0031	0·0281
	Black	2·63	2·50	24·7	23·1		
Relative humidity	Moist	2·38	2·38	22·1	23·4	0·0781	0·0452
	Dry	2·50	2·38				
Degree of crowding	Isolated	2·70	2·25	23·7	23·6	0·2041	0·1227
	Crowded	2·78	2·39				

*Based on the differences between the initial and final ratings.

27·5°C, to various lower and higher temperatures. The groups equilibrated at 18°C were transferred to near-constant temperatures of about 21, 27·5, 32·5, and 35°C, and those equilibrated at 27·5°C to 21, 18·5, 16, and 4°C. Transfers were made over a period of 3 days, by when two groups, or 20 insects, had been taken through each transfer.

Before transfer on each day, the insects that had been equilibrated overnight at each initial temperature (in numbered 1⅞ by ⅝ in. tubes) were ran-

domly divided into groups of 10. Each group was rated and transferred to the appropriate new temperature. The required temperatures were provided by constant-temperature rooms, except the temperature 21°C. To reduce temperature fluctuation, especially at 21°C, which was attained in an unconditioned laboratory, the tubes containing the insects were placed, along with a thermometer, in a large glass tube plugged with cotton wool, which was suspended in a jar of water. The large tube and jar of water had previously been allowed to acquire the temperature of the room. Apart from damping out short-period fluctuations, this arrangement allowed longer-period temperature changes, such as tended to occur particularly at 21°C, to be controlled by adding small quantities of hot or cold water to the water in the jar. However, this arrangement must also have had the effect of accentuating the inevitable time lag between transfer to the new temperature and accommodation of the internal temperature of the insects to it; for the insects were separated from the water in the jar by two layers of glass and two of air. After transfer the insects were rated every ½-1 hr during the initial period of rapid colour change, and then less frequently until equilibrium was attained. At each rating the temperature within the large glass tube was recorded. The experimental temperature was taken to be the mean of these readings, which covered a maximum range, within any one room, of 3°C, and in most rooms of little more than 1°C.

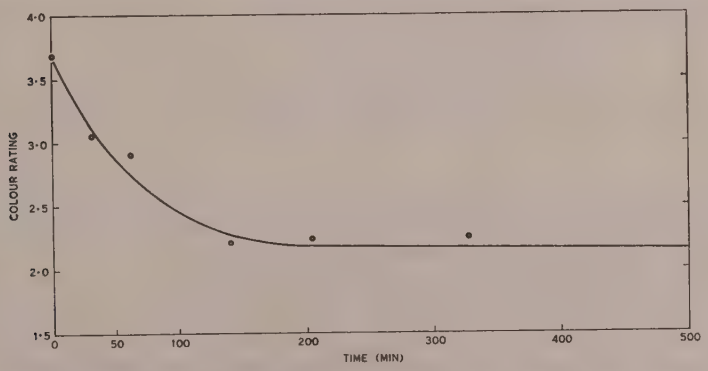

Fig. 1.—Mean colour ratings of a single group of 10 *K. tristis* males, equilibrated at 16·7°C, at time intervals up to 8 hr after transfer to 27·5°C.

The mean ratings for each group of 10 insects were plotted against time and freehand curves were drawn through the resulting points. In this way two independent curves, representing colour accommodation with time, were obtained for each of the eight transfers. A typical plot is presented in Figure 1 to illustrate the goodness of fit. Mean curves were then drawn for each transfer. These are presented in Figures 2 and 3. All figures show only the first 500 min of the plot.

It will be seen that the curves approximate to the exponential form. Whether the change is in the direction of the blue or the black end of the scale, its

rate is at first relatively high, but falls off progressively, the equilibrium rating being approached only very gradually. The latter can be only approximately estimated from the curves. Before transfer, the insects had been held at the initial temperature for periods of 16-20 hr, with an average of 18 hr, and the curves suggest that equilibrium should have been attained by that time. Certainly no changes of consequence occurred after about 18 hr, and the equilibrium rating was therefore taken to be that given by the curves for the time interval of 1100 min.

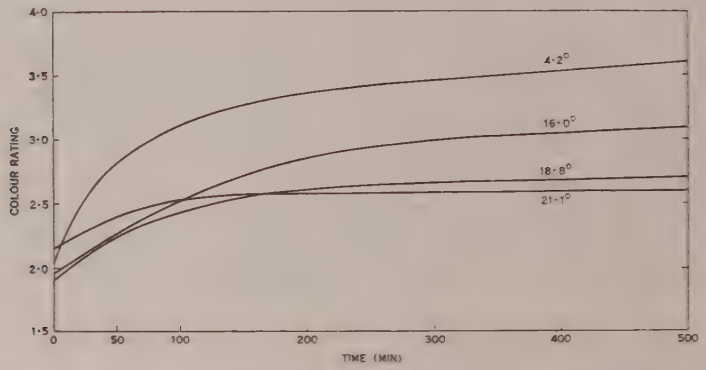

Fig. 2.—Colour accommodation of *K. tristis* males upon transfer from 27·5°C to indicated lower temperatures. Each curve the mean of two curves based upon 10 insects each.

It may be seen from Figure 2 that the rate of colour change within the first hour after transfer is greatest when the transfer is to the lowest temperature employed and progressively less at temperatures differing less from the initial temperature of 27·5°C. This indicates that the rate of change during the early stages of accommodation is governed by the temperature differential rather than by the temperature level itself. This effect may well be reinforced by the lag in the accommodation of the internal temperature of the insects to the new environmental temperature, because this would be relatively greatest where the temperature differential is least. However, the lag in internal temperature accommodation cannot be wholly, or even mainly, responsible. In the case of the insects transferred to 4·2°C, the thermometer in the large glass tube recorded 7·0°C at 32 min after transfer and 3·5°C at 63 min. Even if the mean internal temperature over the first hour had been as high as 16°C, the rating reached at the end of that time was not attained by insects transferred to 16°C (Fig. 2) until over 200 min after transfer, notwithstanding that those insects must by then have held an internal temperature of 16°C for at least 2 hr.

Figure 3 shows the same relation between rate of change during the first hour and temperature differential, but the interpretation here is ambiguous, since the same effect could be imagined to result from the absolute temperature

level after transfer, which in this instance is positively correlated with the temperature differential instead of negatively as in Figure 2.

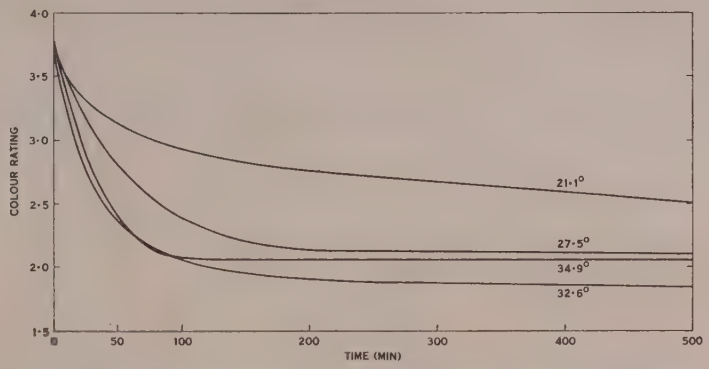

Fig. 3.—Colour accommodation of *K. tristis* males upon transfer from 18°C to indicated higher temperatures. Each curve the mean of two curves based upon 10 insects each.

TABLE 2

MEAN RATE OF COLOUR CHANGE OVER THE FIRST HOUR AND OVER 80 PER CENT. OF THE TOTAL CHANGE, FOR TRANSFERS OF *K. TRISTIS* MALES FROM ONE TEMPERATURE TO ANOTHER, IN RELATION TO THE TEMPERATURE DIFFERENTIAL

Data from the colour accommodation curves

Transfer		Temperature Differential (°C)	Rating Difference at 60 Min	Rating Difference at 1100 Min (*a*)	80% of (*a*)	Time to Reach 80% of (*a*) (min)	Mean Rating Change per Hr Over 80% of (*a*)
From (°C)	To (°C)						
16·7	21·1	4·4	0·75	1·75	1·40	600	0·14
16·7	27·5	10·8	1·03	1·69	1·35	98	0·83
18·0	32·6	14·6	1·43	1·91	1·53	76	1·21
18·0	34·9	16·9	1·44	1·68	1·34	52	1·55
27·6	21·1	6·5	0·28	0·44	0·35	84	0·25
27·0	18·8	8·2	0·39	0·88	0·70	185	0·23
27·6	16·0	11·6	0·37	1·37	1·10	400	0·17
27·6	4·2	23·4	0·86	2·00	1·60	545	0·18

The situation will be made clearer by reference to columns 2-4 of Table 2, and Figure 4. The lower straight line in Figure 4 shows the increase in the rate of change with increasing temperature differential, notwithstanding the lower temperature levels associated with the higher differentials. The steeper slope of the upper line may indicate an accelerating effect of the temperature

level, superimposed on the effect of the temperature differential. The vertical separation of the two lines is of considerable interest. Thus the rate of change of insects transferred to 21·1°C from a lower temperature is nearly three times that of insects transferred from a higher temperature, although the temperature differential in the latter case is slightly the larger. The difference cannot be

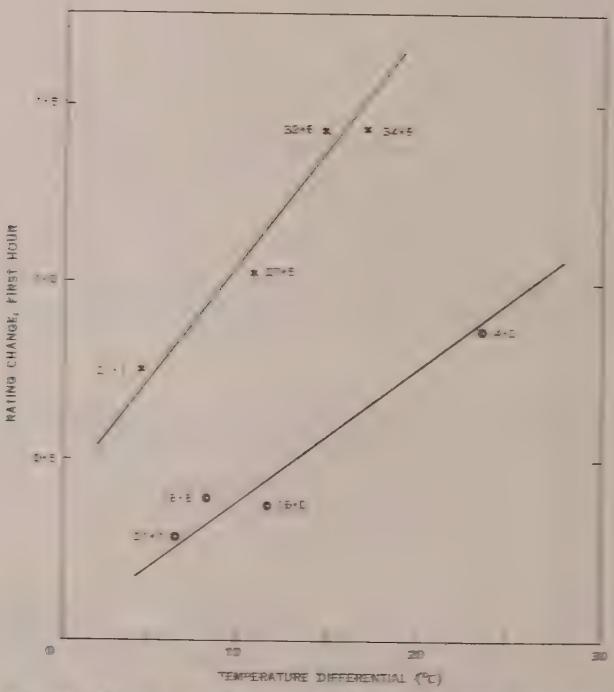

Fig. 4.—Change in colour rating during the first hour after transfer of *K. trusti* males from about 18°C to four higher temperatures (crosses) and from about 27·5°C to four lower temperatures (circles), in relation to the temperature differential. Figures opposite each point indicate the temperature after transfer.

ascribed to the lag in internal temperature accommodation, for that would tend to have the opposite effect. It thus appears that there is a difference in the initial rate of colour accommodation according to whether the temperature is raised or lowered, quite apart from other factors. That is, other things being equal, the change towards blue proceeds more rapidly than the change towards black, a conclusion in line with the indication (Key and Day 1954) of a greater lag between colour rating in the field and the corresponding equilibrium rating when the insects are in process of changing from the blue to the dark phase in the afternoon.

If one considers the mean rate of change over the whole course of the colour accommodation curves instead of over the first hour, the picture is somewhat different. It may be seen from Figures 2 and 3 that the time required to reach a rating near the equilibrium value is in general greater at the lower temperatures, even in the "down" transfers where the temperature differential is greatest at the low temperatures and the rate of change within the first hour most rapid. In view of the difficulty of attempting to define the time at which the equilibrium rating was first attained, attention has been concentrated on

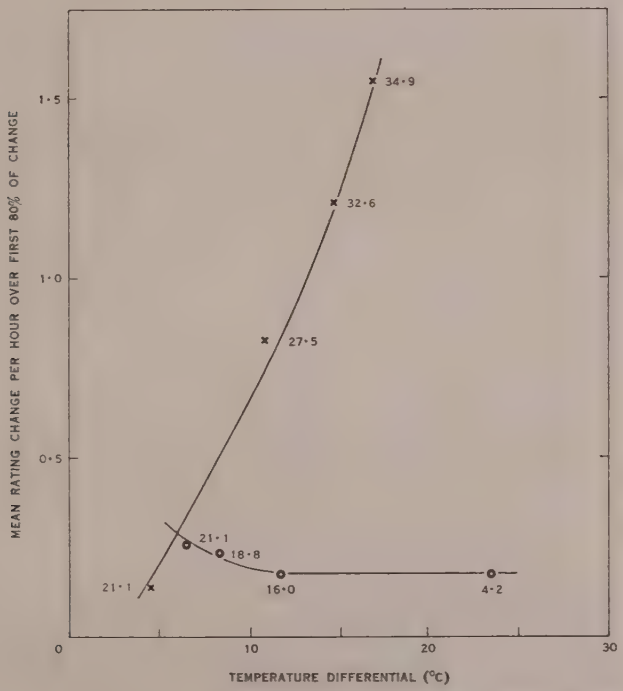

Fig. 5.—Mean change in colour rating per hour over the first 80 per cent. of the change resulting from transfer of *K. tristis* males from about 18°C to four higher temperatures (crosses) and from about 27·5°C to four lower temperatures (circles), in relation to the temperature differential. Figures opposite each point indicate the temperature after transfer.

that portion of each curve over which 80 per cent. of the change took place. The time required to complete 80 per cent. of the change, and the mean rating change per hour over that period, are set out in the last two columns of Table 2. The mean rating change per hour has been plotted against the temperature differential in Figure 5, which should be compared with Figure 4. It may be seen that the relation to temperature differential for transfers to lower temperatures is if anything reversed in comparison with Figure 4, the rate of

change being highest at the low temperature differentials associated with the higher temperature levels. For transfers to higher temperatures, reactions to temperature differential and temperature level are not to be distinguished.

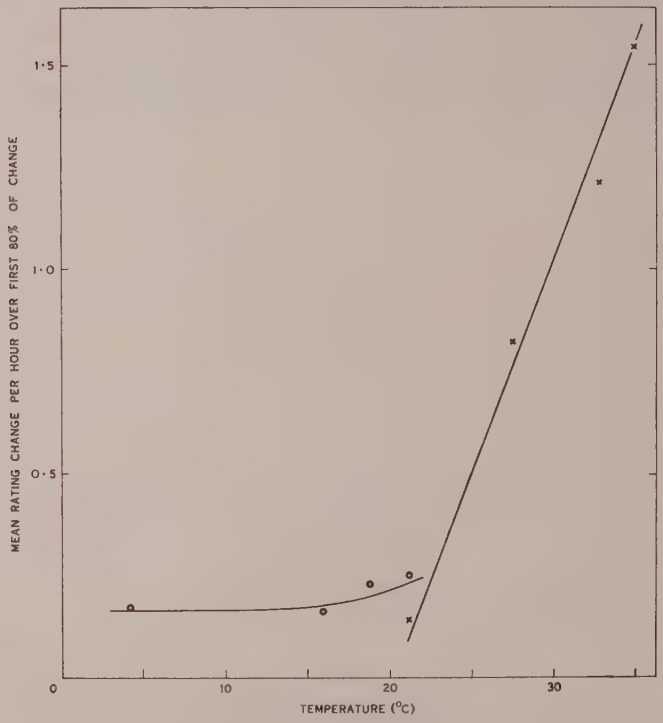

Fig. 6.—Mean change in colour rating per hour over the first 80 per cent. of the change resulting from transfer of *K. tristis* males from about 18°C to four higher temperatures (crosses) and from about 27·5°C to four lower temperatures (circles), in relation to the temperature after transfer.

Thus it would seem that the rate of completion of the colour accommodation, as distinct from the initial rate of colour change, is governed mainly by the environmental temperature rather than by the temperature differential. In Figure 6 the mean rate over 80 per cent. of the change is plotted against the environmental temperature. In this instance the rate at 21·1°C is higher for the "down" transfer than the "up" transfer (cf. Fig. 4); the direction of the difference is such as could be accounted for by the lag in internal temperature accommodation, but it is doubtful whether this factor, operative over at most the first hour after transfer, could produce a difference of this magnitude in the mean rate over 80 per cent. of the change, which in the "up" transfer required 10 hr. In view of the possibility that here, as within the first hour, there may be a fundamental difference between the rates of accommodation

upon transfer from higher and from lower temperatures, separate lines have been drawn through the two sets of points in Figure 6, although the positions of the points themselves would be consistent with a single curve somewhat sharply bent at about 21°C.

TABLE 3

MEAN EQUILIBRIUM COLOUR RATINGS FOR GROUPS OF *K. TRISTIS* MALES EQUILIBRATED FOR 16-20 HR AT VARIOUS CONSTANT TEMPERATURES

Source of Data (Symbols refer to Fig. 7)	Temperature (°C)	Mean Rating	No. of Insects	No. of Ratings per Insect
Rating reproducibility test (Table 7) □	15·7	3·65	10	5
	28·2	1·85	10	5
Reproducibility test with different light sources (Table 8) ×	24·0	2·37	20	8
Transfer experiments: initial ratings	16·7	3·85	30	1
	16·7	3·77	30	1
	19·3	3·58	20	1
	27·0	2·13	30	1
	27·0	1·80	20	1
○	28·3	2·02	30	1
Transfer experiments: ratings after 1100 min (transfer from 18°C)	20·9	2·24	10	1
	21·3	1·91	10	1
	27·1	2·10	10	1
	27·9	2·03	10	1
	32·3	1·94	10	1
	32·8	1·65	10	1
	34·5	2·25	10	1
●	35·2	1·86	10	1
Transfer experiments: ratings after 1100 min (transfer from 27·5°C)	4·1	4·01	10	1
	4·4	4·06	10	1
	15·3	3·49	10	1
	16·7	3·16	10	1
	18·7	2·93	10	1
	18·9	2·63	10	1
	21·1	2·42	10	1
●	21·2	2·75	10	1
Bisection experiment: controls (Table 5) △	4·4	3·88	12	1

The transfers employed in these experiments are not particularly well adapted to an elucidation of the problems of rates of change that they raise. More useful information would be provided by transfers from about 4°C to each

of several higher temperatures, including about 33°C, and from about 33°C to the same temperatures and about 4°C; and by transfers from several different initial temperatures to a common final temperature.

(*c*) *Equilibrium Colour Rating at Different Temperatures*

Figures 2 and 3 indicate that the equilibrium colour rating at constant temperatures varies rather regularly with the temperature, and it has already been noted that equilibrium can be considered complete at all temperatures after about 18 hr exposure. Figures for the equilibrium rating at different temperatures are provided by the rating reproducibility test (Table 7), the test of reproducibility using different light sources (Table 8), the transfer experiments (initial ratings and ratings after 1100 min), and by the intact controls employed in connection with the experiment on isolated halves of the body to be described in Section IV (*b*) (Table 5). These figures, together with the relevant temperatures, are set out in Table 3 and plotted against temperature in Figure 7. The initial ratings from the transfer experiments are means within the six homogenized groups from which groups of 10 insects were drawn for transfer to different temperatures, while the ratings after 1100 min are taken from the accommodation curves for the individual groups of 10 and not from the mean curves.

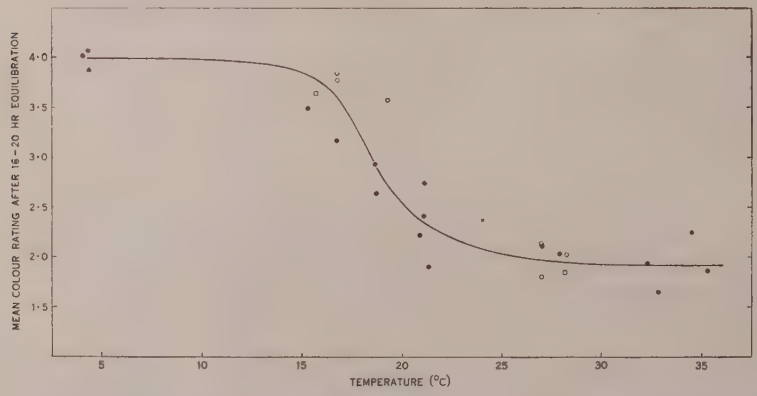

Fig. 7.—Equilibrium colour rating of *K. tristis* males in relation to temperature. Source of data: Table 3.

The equilibrium curve of Figure 7 has been drawn freehand. It is sigmoid in form, flattening out to a rating of 4·0 at low temperatures and about 1·9 at high temperatures. With rising temperature, there is probably little departure from the limiting rating of 4 until 10°C is exceeded, for on the Kosciusko Massif all of 10 rated insects gave this value when the blackened-bulb temperature was 9·7°C and under circumstances that make it most unlikely that the temperature had been lower during the preceding 5 hr (Key and Day

1954). Almost the whole of the difference between the black and blue phases is determined within the temperature range 15-25°C, the sensitivity to temperature differences being greatest at about 18°C, when the rating is about 3. There is no clear evidence of appreciable rating change above about 27°C. The ratings given in Table 3 for temperatures of about 21°C suggest the possibility of a small difference in the equilibrium rating according to whether the previous temperature had been lower or higher, but are far from establishing such a difference.

IV. Experiments on the Physiological Mechanism

The colour change in *K. tristis* could be brought about by one of three broad mechanisms: neural, hormonal, or independent effector, or some combination of these. To elucidate the mechanism responsible, insects were inserted through a cardboard partition and their anterior and posterior halves exposed to different temperatures. Experiments were also carried out in which insects ligated at different places, isolated halves of insects, and isolated portions of integument *in vitro* were subjected to high and low temperatures and their colour response noted.

(*a*) *Response to Localized Cooling and Warming in the Intact Insect*

Two experiments with a cardboard partition were carried out, 10 males being employed in each. Holes were made in the partition just large enough to accommodate the insect's body; the insects were held in position by cellulose tape, the head and the greater part of the pronotum projecting on one side of the partition, and the abdomen on the other side. Five of the insects faced in the one direction and the other five in the other. The partition was formed into one wall of a box, into which an electric light bulb projected. The box was installed in front of the refrigerator fan of a room maintained at about 4°C. With the electric light bulb switched on, a marked temperature differential was maintained between the two sides of the partition. In experiment 1 the temperature of the air on the cold side was about 10·5°C in the position occupied by the insects, and that on the hot side 32°C; in experiment 2 the figures were 6 and 32°C (rising to 34·5°C by the end of the experiment).

The insects in experiment 1 were exposed to the temperature differential for 4·5 hr and the colours of the head and abdomen of each insect then independently rated. In experiment 2 an exposure of 5 hr was given, and ratings were made after 2·3 hr and 5 hr. The results of the ratings are presented in Table 4. It follows from the arrangement of the insects in the partition that the mean ratings for heads on the cold side and abdomens on the hot side refer to the same insects, as do the ratings for abdomens on the cold side and heads on the hot side. It may be seen that, at the conclusion of each experiment, both the heads and the abdomens on the cold side of the partition were very much darker than their fellows on the hot side, the differences in rating being highly significant in every case. Even after 2·3 hr in experiment 2, the differences just attained significance. At both temperatures, the abdomens gave a con-

sistently lower mean rating than the heads, indicating an inherent colour difference between the two parts (see also Key and Day 1954, Plate 1). Nevertheless, at each rating, the diagonal comparison between the abdomens on the cold side and the heads of the same insects on the hot side shows that the abdomens had the higher mean values: the colour change due to the temperature difference reversed the normal colour difference between the head and abdomen. The extent of the difference in rating value due to the temperature differential ($a - b$ in Table 4) is at each time of rating greater for the abdomens than for the heads, but in no case is the difference in response significant; moreover, a pooled test of the interaction gave the same result, a value of 2·55 being obtained for F, while 4·35 is required for significance at the 5 per cent. level.

TABLE 4

MEAN COLOUR RATINGS OF HEADS AND ABDOMENS OF INTACT *K. TRISTIS* MALES FACING IN OPPOSITE DIRECTIONS THROUGH A CARDBOARD PARTITION OF WHICH ONE SIDE WAS BATHED IN COLD AIR AND THE OTHER IN WARM AIR

Experiment No.	Exposure Time (hr)	Part of Body	Mean Colour Rating			S.E. of Difference	Significance of Difference
			Cold Side (a)	Hot Side (b)	Difference ($a-b$)		
1	4·5	Head	3·50	2·40	1·10	0·187	$P<0·001$
		Abdomen	3·40	2·10	1·30	0·212	$P<0·001$
2	2·3	Head	2·63	2·25	0·38	0·168	$P=0·05$
		Abdomen	2·33	1·38	0·95	0·390	$P=0·04$
	5·0	Head	3·00	2·17	0·83	0·230	$P<0·01$
		Abdomen	2·75	1·50	1·25	0·296	$P<0·01$

At the conclusion of experiment 1 the partition was reversed, so that those insects that had previously faced out of the box now faced into it, and vice versa. Further ratings were made 6 hr later. Although four of the insects had died by then, it was quite clear, from the ratings of both living and dead, that both the heads and abdomens on the side that was now cold were darker than when they were hot and darker than the corresponding parts on the side that was now hot; these in turn were paler than when they were cold.

Notwithstanding the imperfect insulation afforded by the cardboard partition, and the fact that heat interchange could take place through the bodies of the insects, these experiments demonstrate that, in intact insects, the colour change can take place in opposite directions in the anterior and posterior portions of the body in response to localized differences of temperature.

(*b*) *Response of Isolated Halves of the Body*

Six males were ligated between the head and pronotum. Three were exposed to 4·4°C and three to about 35°C. After 4½ hr no difference in colour could be detected on the two sides of the ligature in any of the insects. The colour of both groups was approximately that normal for the temperatures to which they were exposed. The same results were obtained with six males ligated at the second abdominal segment.

A further six males were ligated at the second abdominal segment and the abdomen completely severed from the rest of the body by cutting it off in front of the ligature. Three of the severed abdomens were exposed to 4·4°C and three to about 35°C. After 4½ hr both series showed approximately the normal colour response of the intact insect. Ten males, with a mean colour rating of about 2·5 at room temperature, were ligated immediately behind the pronotum, and the abdomen severed behind the ligature. The front halves were divided into two groups of five, and one group exposed to 4·4°C and the other to about 35°C. After 5 hr the group at 4·4°C gave a mean rating of 3·3 and the group at 35°C a rating of 2·4, the difference being highly significant. These ratings are little different from the values to be expected from intact insects after the same period of exposure.

These experiments indicated that ligated insects and isolated halves of the body behave in a closely similar way to intact insects. A more rigorous test was provided by the following experiment.

The abdomens of 23 males, kept at room temperature, were ligated in two places as close as possible to their anterior ends, and the body bisected between the ligatures. The two halves of each insect were associated by appropriate numbering and the insects were randomly divided, as wholes, into two groups designated *A* and *B*. Both groups, along with 20 intact controls, were placed at 4·4°C overnight. The following morning the front halves of group *A* and the abdomens of group *B* were transferred to 35°C, along with a randomly selected group of 10 of the controls. The front halves of group *B* were then rated on the basis of the pronotal disk, and the abdomens of group *A* on the basis of the two or three tergites immediately behind the ligature. The 10 controls that remained at 4·4°C were similarly rated on both pronotum and anterior abdominal tergites. The isolated halves that had been transferred to 35°C were rated in the same way as those at 4·4°C after an exposure period of 70 min and the transferred controls after 80 min.

The experiment permits a comparison between the isolated halves of the same insect when submitted to different temperatures and also between the corresponding halves of bisected and intact insects submitted to the same treatment. The results are set out in Table 5. The ratings for isolated halves of the same insects are diagonally opposite each other in the two temperature columns, whereas in the controls the values for pronotum and abdomen of the same insects are in the same column.

The table shows that the mean colour rating for both the isolated front half and the isolated abdomen was much higher at 4·4°C than at 35°C, the

differences being highly significant. An inherent colour difference between the pronotum and the abdomen is demonstrated for both the isolated halves and the controls, its magnitude being of the same order as that already demonstrated (see Table 4) between the head and the abdomen. Nevertheless, the isolated abdomen at 4·4°C was much darker than the isolated front half at 35°C. In order to determine whether the colour response to temperature was in any way affected by bisection of the insect, the mean rating differences between 4·4°C and 35°C for the isolated front half and the isolated abdomen were compared with the corresponding differences for the pronotum and abdomen of the controls (Table 5). In both comparisons the extent of the response was slightly

TABLE 5

MEAN COLOUR RATINGS OF ISOLATED FRONT HALVES AND ABDOMENS OF *K. TRISTIS* MALES EXPOSED, WITH INTACT CONTROLS, TO 4·4°C OVERNIGHT AND TO 35°C FOR 70-80 MIN

Material	Part of Body	Mean Colour Rating				S.E. of Difference	S.E. of Mean
		4.4°C (*a*)	35°C (*b*)	Difference (*a*—*b*)	Mean (*a*, *b*)		
Isolated halves	Pronotum	4·00	2·42	1·58	3·21	0·056 ($P<0·001$)	0·028
	Abdomen	3·46	1·77	1·69	2·61	0·190 ($P<0·001$)	0·095
Intact controls	Pronotum	3·88	2·19	1·69	3·03	0·151 ($P<0·001$)	0·075
	Abdomen	3·50	1·77	1·73	2·63	0·173 ($P<0·001$)	0·087
Isolated—control	Pronotum	0·12	0·23	—0·11	0·18	0·161 (not sign.)	0·080 ($P<0·05$)
	Abdomen	—0·04	0·00	—0·04	—0·02	0·257 (not sign.)	0·129 (not sign.)

greater in the controls, but the differences were far from significant. If the mean rating for the isolated front half (obtained by averaging the values obtained at the two temperatures) and the corresponding mean for the isolated abdomen are compared with the corresponding figures for pronotum and abdomen in the controls, the abdomen shows no significant difference, but the pronotum is seen to be somewhat darker in the bisected insect, the difference being just significant at the 5 per cent. level. The significance in the last comparison is to be ascribed largely to the exceptionally low variability of the ratings of the isolated front halves, which in turn is partly due to the ratings at 4·4°C having reached the limiting level of 4·0 (see Section II and Appendix I). The difference is quite possibly a population one not related to bisection, since the bisected and control groups were in this instance not drawn from a previously homogenized population. In any case, by the standard of the average equilibrium rating for 4·4°C (Fig. 7), the isolated front halves behaved more normally than the controls, whose rating of 3·88 is decidedly low. A comparison of this kind cannot be made for the ratings at 35°C, since the

period of exposure, kept short to ensure survival of the isolated halves, did not permit equilibrium to be fully attained.

Apart from the single significant difference just discussed, which does not concern the colour difference as between different temperatures, it may be said that bisection of the insects had no effect upon their colour response. It is of interest to note that many of the isolated halves survived at 4·4°C for 3 days, and when these were then transferred to 35°C they went blue in typical fashion.

(*c*) *Response of Isolated Integument* in vitro

The integument of 10 males at room temperature was dissected off from the frons, pronotum (a transverse strip including both disk and lateral lobe), and three tergites in the central region of the abdomen. The pieces from each insect were placed in Griffiths and Tauber's (1943) physiological salt solution (*"Periplaneta* Ringer")* and adhering tissue removed as far as possible. Each

TABLE 6

MEAN COLOUR RATINGS OF ISOLATED INTEGUMENT FROM THE FRONS, PRONOTUM, AND ABDOMINAL DORSUM OF *K. TRISTIS* MALES, AFTER EXPOSURE FOR 30 MIN TO 4·4°C AND ABOUT 35°C IN A PHYSIOLOGICAL SALT SOLUTION

Source	Mean Colour Rating			S.E. of Difference	Significance of Difference
	4·4°C	35°C	Difference		
Frons	3·50	1·70	1·80	0·283	$P<0·001$
Pronotal disk	3·65	2·50	1·15	0·198	$P<0·001$
Abdominal tergites	3·15	2·22	0·93	0·216	$P<0·001$

piece was then divided into two along the mid line, and one half placed in salt solution maintained at 4·4°C, the other half at about 35°C. Appropriate labelling of the dishes of solution enabled all the pieces from any one insect to be associated. Each piece of integument was rated after 30 min. The mean ratings for the 10 insects are set out in Table 6.

It may be seen that the pieces of integument maintained at 4·4°C were very much darker, after only 30 min, than their opposite halves maintained at 35°C, the difference in the mean rating being highly significant for each region of the body examined. Further ratings of a few pieces were made after 3 hr; comparison with the ratings after 30 min showed that a recession in the level of colour accommodation had occurred, the tissue having presumably begun to degenerate.

The results of the above experiments on the localized cooling and warming of the intact insect, on the behaviour of ligated insects and isolated halves of insects, and on the isolated integument *in vitro* afford not the slightest ground

* NaCl, 14·0 g; $CaCl_2$, 0·4 g; KCl, 0·2 g; $NaHCO_3$, 0·2 g; made up to 1 l. with distilled water.

for supposing that a coordinating mechanism of any kind is involved in the colour response of *K. tristis*. The experiment *in vitro* is quite decisive by itself and indicates that the epidermis acts as an independent effector organ. Although it is conceivable that there may be some differentiation of function, in relation to the colour response, between the component cells of the epidermis, there is no apparent histological evidence of this. Further, Slifer (1953) has found that *K. tristis* is the only species of acridid, of 122 studied by her, in which thermoreceptors (see also Slifer 1951) appear to be *absent* from the integument. Thus the probability is that each individual epidermal cell is an independent effector, and that the whole of the mechanism of the colour response must be sought in intracellular processes.

V. Discussion

Physiological colour change brought about by the activity of independent effectors is not common among animals, and is rare in invertebrates (see Prosser 1950). Usually a nervous or endocrine mechanism is involved, or both. However, where, in either vertebrates or invertebrates, a colour response to temperature exists, it is apparently most commonly due to independent effector activity (cf. Brown and Sandeen 1948). Among insects, physiological colour change of any kind, except for pigment movement in the compound eyes (cf. Dethier 1953), has rarely been reported, the only well-known examples being the phasmid *Carausius morosus* Brunn. & Redt. (Giersberg 1928 and others) and the larva of the chironomid *Corethra plumicornis* (Fabr.) (Kopenec 1949). More than one conditioning factor can lead to colour change in both of these insects, and for most of the responses a nervous-endocrine mechanism has been established. However, Kopenec (1949), with reference to the darkening effect of bright light on *C. plumicornis* larvae, states that "es handelt sich hier wahrscheinlich um eine direkte Wirkung auf die Melanophoren"; and Giersberg (1928) found that temperature, narcosis by ether, and osmotic influences produced their effect in *Carausius morosus* by acting as a direct stimulus on the epidermal cells of the integument.

Carausius morosus is the only animal in which a colour response closely comparable to that of *Kosciuscola* has been reported and, as far as the authors are aware, the only paper dealing directly with that response is the one of Giersberg (1928). Although in this insect the primitive direct response to temperature overrides the other colour-change mechanisms, the attention of later authors seems to have been concentrated upon the mechanisms involving coordination, and Wigglesworth (1950), Prosser (1950), and Roeder (1953) do not refer to colour change due to independent effectors in this or any other insect. Janda (1935), however, put out of action the mechanisms involving coordination in dark forms of *C. morosus* by the use of ligatures, and found that that part of the body behind the ligature reacted to temperature only, remaining dark at temperatures up to 12°C and becoming pale most rapidly at 33°C. These figures are in close agreement with those of Giersberg.

The essential features of correspondence between the temperature response of *C. morosus*, as described by Giersberg, and that of *K. tristis* are:

(1) "High" temperatures (30-32°C) produce a paling, and "low" temperatures (0-12°C) a darkening;*
(2) Temperature acts directly on the effector organ without coordination of any kind being involved;
(3) The effector is the unspecialized epidermal cell, in which migration of pigment granules takes place;
(4) The change is visible within half an hour; and
(5) The effect of high temperature is "deutlicher" (i.e. "clearer," but perhaps meaning "more rapid") than that of low temperature.

Giersberg produced alternating bands of pale and dark integument along the length of the very attenuated body of *C. morosus* by passing warm and cold water through glass tubes straddling the animal and in contact with its integument (Giersberg 1928, Fig. 7). This experiment corresponds to the cardboard partition experiment performed on *K. tristis* (Section IV (*a*)). He also demonstrated a persistence of the response in narcotized insects.

Interesting parallels between the colour response of *K. tristis* and that of an animal as far removed as a vertebrate equipped with melanophores are revealed in the work of Parker (1938) on the lizard *Phrynosoma blainvillii* Gray. In this animal a direct, localized response of the melanophores produces pallor at temperatures of about 37°C and darkening at 3-6°C.

The absence of any coordinating mechanism in the colour change of *K. tristis* brings into sharp focus the problem of the intracellular mechanism responsible for the movement of granules within the cytoplasm—although the same problem arises eventually where nervous or endocrine coordination is involved. Any attempt at a solution will need to take into account the character of the movement of the two types of granule (Key and Day 1954), the influence of the temperature differential on the rate of colour accommodation during its early stages and of the absolute temperature during the later stages (Section III (*b*)), the influence of the direction of transfer upon the rate of accommodation, i.e. the greater rapidity of the change from black to blue (Section III (*b*)), and the relation between the equilibrium distribution of the granules and temperature.

The most striking observation in this connection is the simultaneous movement of two types of granules in opposite directions within the cell. This fact, along with the absence of any evidence that the granules follow definite flow-paths, seems to exclude an explanation based on cytoplasmic streaming. It seems more likely that the granules make their way *through* the cytoplasm in response to some kind of gradient or "field." This is especially suggested by the behaviour of the blue granules, which seem to move in a very individual manner, being gradually released from their attraction to a "pole" at one end of the cell and slowly accumulating at the opposite "pole" (see Key and Day 1954). On this interpretation, the temperature differential may be supposed to deter-

* The temperature threshold for the commencement of paling and the optimum temperature for paleness are both extraordinarily close to the corresponding temperatures for *K. tristis* (see Section III (*c*)).

mine the initial intensity of the "field" (which could not, of course, be simply a temperature gradient), while the absolute temperature might govern the rate of completion of the accommodation by its influence on the viscosity of the cytoplasm. It is not easy to reconcile this type of scheme with the stable equilibrium distribution of the granules in intermediate positions at temperatures towards the middle of the effective range. The problem obviously calls for the attention of specialists in the biochemistry and biophysics of the cell, and it is possible that *K. tristis* may prove a useful laboratory animal for such workers, because of the absence of any mechanism of coordination superimposed on the reactions of the cell itself and the possibility of eliciting the response *in vitro*.

VI. Acknowledgments

The authors are greatly indebted to Mr. G. A. McIntyre, Section of Mathematical Statistics, C.S.I.R.O., for assistance in the evaluation of the experimental data. Mr. L. A. Marshall, Division of Entomology, C.S.I.R.O., supervised the preparation of the graphs, and Mrs. M. J. Bennetts assisted in the conduct of the experiments.

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Appendix I

Analysis of reproducibility of colour ratings

The reproducibility of the ratings was investigated in the first place on two groups, each of 10 males, which were held overnight in constant-temperature rooms at mean temperatures of 28·2 and 15·7°C respectively. Each group was rated, in random order, on five separate occasions during the following day. The tubes were numbered, so that the successive ratings for each individual could be compared. The mean ratings and analysis of variance are presented in Table 7. During the course of the observations on the group at 15·7°C there was a fall in temperature from 16·1 to 15·4°C and this may account in part for the relatively large variance between occasions. Assuming that the effect would have been linear, the residual mean square allowing for such effect is 0·1175, which is significant ($P < 0·05$), indicating that some variation in mean rating can occur between occasions. Pooling the results for the two groups and allowing for the temperature change in the group at 15·7°C, the pooled mean square between occasions is 0·0718 and the interaction variance 0·0305. The estimate of the variance between means for occasions in very large samples is then $1/5\ (0·0718 - 0·0305) = 0·0082$, which is of very little consequence.

Table 7

RESULTS OF A TEST OF REPRODUCIBILITY OF COLOUR RATINGS, MADE BY RATING, ON FIVE SEPARATE OCCASIONS, TWO GROUPS OF 10 MALES EACH, MAINTAINED AT MEAN TEMPERATURES OF 28·2 AND 15·7°C RESPECTIVELY

Temperature (°C)	Mean Ratings on Five Successive Occasions					Mean
28·2	1·80	1·85	1·80	1·85	1·95	1·85
15·7	3·55	3·50	3·75	3·70	3·75	3·65

Analysis of Variance (Mean Squares)

	Between Occasions	Occasions × Specimens
28·2	0·0375	0·0319
15·7	0·1375	0·0292

Since it appeared possible that, whatever the effect of light on the actual colour of the insect, the differences in the quality of the light in the different rooms where rating had to be carried out might introduce a subjective error in matching the colour, the following test was carried out. Twenty males were placed in individually numbered tubes and allowed to equilibrate for 1 hr in

the room in which they had been kept for some days previously, the temperature of which fluctuated between 20 and 24°C. They were then rated, in random order and without intermission, under four different lights, namely, fluorescent "natural," fluorescent "white," incandescent, and daylight, these being the types of light under which rating was carried out in the main experiments. All 20 were rated under one light before passing to the next, two complete sets of ratings being made under each light in the following order: daylight 1, fluorescent "natural" 1, incandescent 1, daylight 2, fluorescent "natural" 2, incandescent 2, fluorescent "white" 1, fluorescent "white" 2. All the artificial lights were of 40 W and frosted. The results of the test are presented in Table 8.

TABLE 8

RESULTS OF A TEST OF REPRODUCIBILITY OF COLOUR RATINGS UNDER DIFFERENT QUALITIES OF INCIDENT LIGHT: MEAN RATINGS OF 20 MALES, EACH RATED ON TWO SEPARATE OCCASIONS UNDER EACH OF FOUR DIFFERENT LIGHTS

Rating	Fluorescent "Natural"	Fluorescent "White"	Incandescent	Daylight
First	2·37	2·37	2·37	2·40
Second	2·40	2·40	2·35	2·30
Mean	2·39	2·39	2·36	2·35

Analysis of Variance (Mean Squares)

Lights	0·0140
Specimens × lights	0·0197
Occasions within lights	0·0280
Specimens × occasions within lights	0·0232

The evidence of Table 8 indicates no significant effect of light on the ratings, at least within the range of quality and intensity employed in the experimental work, nor any greater interaction between specimens and light than between specimens on two occasions under the same lighting.

In the experiments described in Section III (*a*), certain factors other than temperature were found to have no significant effect on colour. The initial and final ratings from these experiments can thus be used to provide further evidence on the reproducibility of the ratings of individual insects relative to one another (interaction variance). However, there was in no instance a certainty that the conditions precluded a change of colour within any group during the course of the experiment, so that no conclusions can be drawn on systematic shifts in judgment between occasions. The interaction variances for these experiments were in general higher than those set out in Tables 7 and 8.

The pooled variance from all relevant data on reproducibility is 0·0322; that is, the standard error of the rating of an insect due to variation in judgment, but excluding systematic shifts in judgment, is 0·179.

From the tests summarized in Tables 7 and 8, together with the experiments on factors other than temperature referred to above, one can determine also the variance between individual insects (specimens), based on a single rating per specimen. The average variance associated with mean ratings of 2 to 3 is of the order of 0·13, or 0·10 if the subjective error of rating is eliminated. For this range, therefore, the variability between specimens is more than twice the error of rating. However, at a mean rating value approaching 4, it is clear from the data on temperature accommodation that the variance between insects decreases sharply, reaching zero in groups in equilibrium with temperatures of the order of 4°C. The difference in variance between the lower and higher rating ranges is in large part due to the rigidly terminal character of the dark colour at low temperatures. No degrees of this "black" can be discerned, and all insects seem to be able to reach the extreme condition after sufficiently long retention at a sufficiently low temperature. The blue colour is not terminal in the same sense: both bluer and paler shades could readily be perceived if the insects exhibited them. The rating value of 1 is not commonly met with, and means for series of 10 or more insects do not fall below 1·6. A further source of variability at the lower ratings is the occurrence of the greener type of male referred to by Key and Day (1954). The colour of this type is not readily matched on the rating chart at ratings below 3, although in the darkest condition it is not distinguishable from the normal type. The subjective error of rating also undoubtedly decreases as the majority of insects come to be rated as 4, and it, too, becomes zero in groups in equilibrium with temperatures of the order of 4°C.

THE FUSION OF PARALLEL LONG BONES AND THE FORMATION OF SECONDARY CARTILAGE

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[*Manuscript received April* 23, 1954]

Summary

The formation of cartilage is described in the repair of the fractured fibula of the guinea pig. Cartilage and bone developed from a common blastema and transitions occurred between them. No convincing evidence was found that cartilage was ever directly transformed into bone.

In some guinea pigs, whether with the fibula fractured or not, the tibia and fibula came into contact near the distal end, and the apposed periostea fused and chondrified. Later the cartilage was resorbed from below and replaced by bone. Thus, the tibia and fibula were united by a bony connection near their distal ends. In the chondrification of the periostea, the fibrous layer was involved as well as the cambial layer. In some specimens the periostea chondrified without fusing, making a structure resembling a nearthrosis.

In the normal development of the rat, the tibia and fibula become fused near their distal ends within a few days after birth. The fusion process begins in the periostea, which unite. The cambial layers then chondrify, and the chondrification process spreads through the fused fibrous layers so that the two bones are united by a cartilaginous pad. This pad is later replaced by bone from below so that a bony union is established.

In the embryo chick, metatarsals 2, 3, and 4 are at first separate cartilages. Periosteal bone develops around each cartilage and at first there is no connection between the three bones. Later, bony trabeculae form on the anterior and posterior aspects, connecting pairs of adjacent bones, close beneath the common fibrous periosteum. The fusion thus occurs without the formation of cartilage.

In the frog, the tibia and fibula, and the radius and ulna, become fused during metamorphosis. No cartilage is formed during the fusion, the two bones becoming enclosed in a common sheath of periosteal bone which develops beneath the common fibrous periosteal layer.

In a discussion of the induction of cartilage formation it is concluded:

(1) That presumptive cartilage cells of the embryo chondrify independently of mechanical stimulation, the determining agent being presumably humoral;

(2) That cells which do not normally chondrify, but which belong to the skeletal group, form cartilage if they are subjected to certain mechanical conditions, and that these conditions include pressure and shear;

(3) That cells of the non-skeletal connective tissue group can form cartilage only if they undergo an induction, presumably by a humoral agent, and that no further mechanical stimulation seems then to be required.

I. Introduction

In work, some of whose results have been published elsewhere (Murray and Kodicek 1949*a*, 1949*b*, 1949*c*), it was noticed that in guinea pigs with

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experimentally fractured fibulae it occasionally happened that the tibia and one of the two pieces of the fibula became fused. The histology of this fusion is of interest and is described in the present paper. Since the fusion involved the formation of secondary cartilage, and since secondary cartilage also formed at the site of the fracture (callus cartilage), some description is here given first of the cartilage formed at the site of fracture and then of that formed at the site of fusion. Studies were made to compare the process of fusion of the tibia and fibula of the guinea pig with the normally occurring fusion of certain long bones of other animals:

(1) Fusion of tibia and fibula in the rat;

(2) Fusion of the metatarsals in the chick; and

(3) Fusion of the tibia and fibula, and radius and ulna, in the metamorphosing tadpoles of an unnamed frog. Unfortunately the systematics of Australian tadpoles have not been studied and the stock of tadpoles died before metamorphosis, so we have not been able to identify our anuran material. It was probably a species of *Crinia*.

II. Methods

For histological material, serial sections were made, both transversely and longitudinally when appropriate, and when material was available. The fixative was Susa and as routine stains Ehrlich's haematoxylin and eosin and Heidenhein's azan were used, and for special purposes Besson's modification of Nicolle's carbol thionin (Carleton 1938, p. 113) and Wilder's silver method for connective tissue fibres.

III. The Guinea Pig

(*a*) *The Cartilage of the Fracture Callus*

Stages in the repair of the fractured fibula were studied in 34 dietetically normal animals which died or were killed at the following numbers of days after operation (in brackets the number of animals): 1-7 (1 animal each), 9 (2), 10-14 (1 each), 15 (4), 16 (4), 64 (1), 81 (3), 84 (4), 94 (3), 101 (1).

The fractures were inflicted without open operation as described elsewhere (Murray and Kodicek 1949*a*).

The single animal killed 1 day after the operation showed no histologically recognizable processes of repair (Plate 1, Fig. 1). There were few or no mitoses in the periosteum, and no accumulation of callus-forming cells at the fracture. In the 2- and 3-day specimens, the onset of repair was shown by the presence of mitoses in the periosteum, and by its more richly cellular nature. There was no accumulation of cells at the callus and no new bone in the fracture region, but in places back from the area of actual injury some very young bone-like tissue had appeared on the surface of the old bone. At 4 and 5 days there was still no considerable cellular accumulation, and no formation of cartilage, at the fracture site.

Histologically recognizable cartilage appeared first in one of the specimens killed 9 days after the operation, but its formation was foreshadowed in the

7- and in the other 9-day specimens. In these, the fractured ends of the bone showed the beginnings of osteoclastic resorption and, in the 7-day specimen especially (Plate 1, Fig. 2) were embedded in a mass of cellular tissue. In the 7-day specimen particularly this tissue tended to have a trabecular structure; the cellular trabeculae were evidently an early stage in the development of the bony callus. In addition, parts of the cellular tissue close beneath the fibrous layer of the periosteum, at the level of the fracture, were arranged massively instead of in trabeculae and (from comparisons with later specimens) were probably about to give rise to cartilage.

Cartilage was present in the callus of one of the 9-day specimens and of most (perhaps all) later specimens up to those killed at 16 days. In the 9-day specimen there was a mass of young cartilage on the tibial side of the fracture. It lay beneath the fibrous layer of the periosteum, which was here continuous over the fracture region and, like the whole of the callus, was developed in the much enlarged cambial zone, and also from the deeper cells of the fibrous layer. Above and below, the cartilage lay between trabeculae of the bony callus, now in active development, and some of the trabeculae merged into the cartilage matrix. This was better seen in an animal killed at 11 days (Plate 1, Fig. 4). The cells of the cartilage were large by contrast with the neighbouring connective tissue cells, had oval vesicular nuclei and dense cytoplasm, and lay in thin-walled basophil "capsules" (Plate 1, Fig. 5, a 9-day specimen). Between the capsules was the rather sparse inter-capsular matrix which tended to be acidophil rather than basophil and which was delicately fibrillar. The cartilage passed by gradual, but rather abrupt, transition into three other tissues: the local connective tissue of the widened cambial zone, the bone of the callus, and, as mentioned above, the deeper parts of the fibrous layer of the periost. There was also in this specimen, on the opposite side of the fibula, some much more advanced cartilage in process of resorption.

There can be no doubt that the cartilage was derived from the massive cellular tissue already noted as present, in earlier stages, at the site of its future formation, and since it still showed, soon after its formation, transitions into the cambial connective tissue, it was probably still being formed from this, by apposition at its surface. Study of the zone of transition from connective tissue to cartilage showed: (*a*) an enlargement and rounding up of the cells, (*b*) the formation of the cartilage cell capsules, and (*c*) the appearance of the inter-capsular matrix (Plate 1, Fig. 6). In this last, one can recognize histologically a diffuse apparently structureless material which stains very slightly with either haematoxylin or eosin but a deep red-purple with Giemsa (Plate 1, Fig. 4), its density increasing towards the middle of the cartilage mass, and having collagen fibres embedded in it.

Discussion of the relation between the developing cartilage and the developing bony trabeculae requires a preliminary word about the early development of the bone. Like the cartilage, it formed from the cellular tissue seen in the earlier stages of the callus (6-8 days in the present material), but from its trabecular portion. The first sign of bony matrix was the appearance in the neighbourhood of the cells of numbers of small granules which were rather

weakly basophil with haematoxylin but stained brilliantly red-purple with Giemsa. These granules were mentioned in an earlier publication (Murray and Kodicek 1949*b*) and have been studied by other authors (e.g. Weidenreich 1930), who tend to regard them, probably correctly, as sites of early deposition of mineral salts. At these sites the organic matrix of the developing bone is supposed to have been altered in such a way that the sites appear as granules even after decalcification. Later the granules seemed to become compacted into solid trabeculae, and true bone, with osteocytes and a non-granular matrix, was deposited upon them. Now certain of the cellular trabeculae which were the precursors of the bone were continuous with the histologically similar but more massive precursors of the cartilage, so that the bony trabeculae continued into the cartilage. As other authors (e.g. Pritchard and Ruzicka 1950) have frequently observed, there was a histological continuity between cartilage and bone with a transition of one tissue into another. In such transition areas (Plate 1, Fig. 6), the bony character of the matrix could often be recognized and traced for some distance into the cartilage by the presence of the granules, while the cartilaginous nature of the tissue was emphasized by the presence of the diffuse-staining cartilage matrix. Thus processes of bone and cartilage formation interpenetrated one another and enable us to understand how it is that in later stages it is often so difficult to draw a sharp line between them.

Appearances such as those just described were often consistent with a direct transformation of cartilage into bone, such as has recently been described in the fracture calluses of the lizard and frog, but not in the rat (Pritchard and Ruzicka 1950), and as has been reported in tissue culture by Fell (1933) and Roulet (1935). I have seen nothing in my preparations which convinced me that such a transformation occurred, the histological picture being always equally consistent with the theoretically more probable differentiation of the two tissues from different parts of a common blastema (an event also described by Pritchard and Ruzicka 1950; Fell 1933; and others).

In later specimens than 9 days, suitably oriented longitudinal sections usually showed two such nodules. Each piece of cartilage lay in the widened cambial zone and often in contact with the fibrous layer of the periosteum, and suggested a cushion between the bony trabeculae running into and merging with it from above and below. The appearance of such sections suggested very strongly that the cartilage acted as a cushion taking up pressures transmitted to it through the bony callus and, by the familiar extension of this line of reasoning, that its formation was elicited by such pressures. That the cartilage probably did develop under pressure was made extremely probable by the general picture of these early stages in the repair process.

In the first days after the operation, the broken ends were in contact (Plate 1, Figs. 1 and 2) and such dead tissue as lay between them was obviously under pressure. But soon the necrotic bone began to be resorbed from the broken ends, and at the same time the first new bone and the cartilage of the callus were forming. Trabeculae of bone formed above and below the fracture site but at first without crossing the gap and meeting one another. Between the

two developing trabecular systems appeared the cartilage, like a cushion. After the resorption phase, the fractured ends were no longer in contact and healthy tissue between them showed no sign of being under pressure (Plate 1, Fig. 4); if such pressure had existed, the broken ends should surely have been in contact. The relief of the pressure was evidently to be attributed to the callus. It should be remembered that the fractured fibula was splinted by the tibia.

Soon after its formation, further changes brought the cartilage to its maturity. The cells enlarged further, the capsules became obvious, and the intercapsular matrix more massive (Plate 1, Fig. 7). The cytoplasm of the cells became intensely vacuolated.

There was little trace of any regularity in the arrangement of the cartilage cells and, except where that role was played by the fibrous layer of the fibrillar periosteum, it was without a perichondrium, for at every surface it passed over either into bone (Plate 1, Fig. 9), into the delicate inter-trabecular connective tissue of the callus (some of whose cells might still be adding themselves to the cartilage), or into the fibrous layer of the periost.

The cartilage attains to the condition just described at about 12 or 14 days.

In its latest stages, the cartilage showed degenerative changes: the nuclei became pycnotic and of irregular form, and the vacuolation which began in the peripheral parts of the cytoplasm extended all through it. Even before degeneration began, resorption of the cartilage set in; indeed resorption might go on at one surface while at another the cartilage seemed still to be growing. The resorption occurred from connective-tissue-covered surfaces and involved the presence of multinucleate osteoclast-like cells which it was presumably correct to call chondroclasts (Plate 2, Fig. 10).

During its degenerative phase, the cartilage was partly walled in by bone and partly resorbed. In some of the 15- and 16-day material it was evident that most of the cartilage had been removed, but clear traces and remnants of cartilage could be found even in some of the 64-day and older material. In these, the fracture callus had been consolidated and what little of the cartilage remained consisted of bits of matrix, sometimes with a necrotic cell or two, walled in by bone and so saved from destruction.

There was a good deal of variation in the times after fracture at which cartilage formation, maturity, and resorption occur. In this material, the cellular callus was present at 6, 7, and 8 days; cartilage was seen first at 9 days, and young to mature cartilage was present at 10 and 11 days. Mature cartilage, with large cells showing vacuolation of the cytoplasm, was present up to 16 days, but resorption had already set in by 12 days. At 64 days and later, only "built-in" remnants of the cartilage remained.

(*b*) *Formation of Cartilage at Surfaces of Contact between the Tibia and the Fibula in the Guinea Pig*

In the guinea pig, the lower ends of the tibia and fibula lie in contact with each other, or at least very close together, and in some animals the two bones were found to be united by a pad-like mass of cartilage. It was at first thought

that this occurred only in legs with fractured fibulae, and that the condition was due to a shift in the lower fibular fragment bringing it into closer contact with the tibia, or to its becoming free to move on the tibia; but the hypothesis was rendered untenable when five unoperated legs were investigated and cartilage pads were identified in two, and less certainly in another. It is, however, true that cartilage only appeared where the two bones were in contact.

Whatever relation the fracturing of the fibula may have had to the development of the cartilage, when the material was studied in order of increasing times since the operation it was found to be arranged in an obvious sequence of advancing stages. This order was probably also approximately the order of increasing age.

A group of three specimens from animals killed 1, 4, and 5 days after operation may be considered together, all being in an early stage of development of the cartilage pad. In the longitudinal sections the cartilage appeared in each as an elongate pad with its base resting against the smooth surface of the bone and occupying the position of the two periostea and of any non-periosteal connective tissue which there might have been between them. The matrix was only very slightly basophil.

In one of the animals killed 24 hr after the operation, chondrification had begun in both periostea (Plate 2, Fig. 11). In the cambial layer of the periost on the tibial side, cartilage matrix was present and the cells had begun to round up, but they lacked capsules and some were still elongate or branching. The fibrous layer of the periosteum, though beginning to be affected by the chondrification, remained readily traceable into the unaffected regions proximal and distal to the chondrifying zone. In the fibular periosteum, the differentiation into fibrous and cambial zones was less clear and chondrification seemed to be beginning in the superficial rather than in the deep layers of the periost. The specimen from the animal killed 4 days after the operation differed from this only in minor details; in that from the animal killed at 5 days chondrification was slightly more advanced and it was clearer that cartilage formation, after beginning in the cambial zone, was spreading thence to the fibrous layer. The same was in general clear in the two specimens from animals killed at 14 and 16 days; but in places the change seemed to begin in the fibrous layer. The cartilage cells were now acquiring capsules which reacted positively with thionin.

During the remainder of the development of the cartilage its matrix became more basophil and the capsules more obvious, both with haematoxylin and eosin, and with thionin; in some of the more advanced specimens, the positive reaction to thionin extended into the matrix between the capsules. The fibrous layers of the periostea, whether fused or in contact without fusing, chondrified right through. In the former more usual case the two bones are joined through a common cartilaginous pad (Plate 2, Fig. 14), while in the latter they remain separate and the two cartilage pads seem to form a structure suggestive of a nearthrosis (Plate 2, Fig. 12).

It was found in the guinea pig (Murray and Kodicek 1949*b*) that fracturing the fibula, or even attempting to fracture it, frequently induced the formation of trabecular sub-periostal reaction thickening of both tibia and fibula, and that in animals partially deficient in vitamin C these thickenings might become enormous. In the specimens described above the new cartilage evidently developed before any such bony thickenings were formed; it formed on flat surfaces of uneroded, non-trabecular bone and as a flat eminence upon it. In certain of the specimens from animals which were not killed so soon after the operation, the cartilage evidently developed after the formation of the reaction thickening and, from the histological picture, apparently as a result of the two sets of trabeculae meeting and compressing the periostea between them. Plate 2, Figure 13, shows the original surfaces of the tibia and fibula separated by a considerable distance; but the intervening space is occupied by a mass of new bone developed on each of the two elements in reaction to the fracturing of the fibula and to the partially vitamin-C-deficient diet (Murray and Kodicek 1949*b*). The cartilaginous pad shown in the photograph developed in the fused periostea; its real superficial extent is much greater than the photograph suggests. In other specimens again the cartilage formed on each bone lay in a depression on its surface; older specimens showed that the cartilage might come to be in a depression because during its formation it took the place of bone formation in the reaction thickening and developed to a thickness not less than that of the bone which it replaced.

In histological character the cartilage of the pad does not differ greatly from that seen in the fracture callus, except that there seems to be a lesser tendency to vacuolations of the cells (Plate 2, Fig. 15).

The fact that in the 1-, 4-, and 5-day specimens the cartilage developed on surfaces which showed no histological signs of injury does not alter the evidence earlier adduced (Murray and Kodicek 1949*b*) that the reaction thickening is a response to injury in the sense of detachment of the periosteum from the bone, and a widening of the cambial zone. Such a situation has much in common with the situation at the site of a fracture. At a fracture, there is evidence that cartilage is formed under pressure, and the cartilage at present under discussion appears very possibly to be related to pressure, or to friction, between the tibia and fibula.

Having been formed, resorption of the cartilage began, blood vessels and connective tissue attacking it from spaces in the underlying bone. Its replacement by bone began (in one 85-day and one 93-day specimen) in the unchondrified but fused periostea at the proximal and distal ends of the cartilage pad, and probably all round its edges. Later, as seen in an 81-day specimen, the resorption process broke up the cartilage into irregular fragments upon which bone was deposited, the bone derived from the tibia uniting with that of the fibula. Our most advanced specimen (Plate 1, Fig. 16) shows a wide bony bridge connecting the two elements.

IV. The Fusion of the Tibia and Fibula in the Rat

Plate 3; Plate 4, Fig. 25

In the adult rat, the tibia and fibula are of very unequal thicknesses and are fused together distally. The slender fibula lies posterior and lateral to the stout tibia; proximal to the fusion area, it is separated from the tibia by a wide interval. Distal to the fusion area, it is again free, and has its own articulation with the tarsus, separate from that of the tibia and lateral to it. The fusion area itself is comparatively short, approximately 7 mm, where the length of tibia and fibula is approximately 30 mm. Sections of the fusion area show that the marrow cavities of the two bones are confluent; and the two bones are in this region invested by a common periosteum.

Transverse and longitudinal serial sections were studied of material from rats of the following ages after birth: 1, 3, 4, 6, 7, 10, and 12 days, and a longitudinal series at 5 days.

(*a*) *One-day Rat*

On the first day the hard tissues of the tibia and fibula were separate from one another, but distally the two bones approach one another and the two periostea coalesce (Plate 3, Fig. 18). The coalescence was restricted to the fibrous layers, so that when the bones approached one another most closely they were separated by two cambial layers and a single (fused) fibrous layer. Further distally, the two periostea became distinct again.

(*b*) *Three-day Rat*

In this material cartilage formation had begun in the region in which the fibrous periosteal layers had coalesced. It first appeared as two strips, one on the postero-lateral aspect of the tibia and the other on the antero-lateral aspect of the fibula, that is, in the angle between the two bones on the lateral aspect of the limb, and not, as one might expect, exactly between the two bones. No cartilage had yet formed in the angle on the medial side.

The first cells to chondrify were the deeper cells of the cambial layers of the two periostea. These cells became enclosed in a nearly homogeneous but faintly fibrillar matrix which stained very weakly with both eosin and the aniline blue of Azan. Nothing, except knowledge of later stages, suggested that this tissue was cartilage rather than uncalcified young bone; it developed in continuity with the previously existing indubitable bone of the tibia and fibula, and peripherally its matrix faded out in a mass of cells which could be either osteoblasts or chondroblasts.

It is noteworthy that the cartilage strips were separated by the whole thickness of the fibrous layers of the two periostea and by the connective tissue between them.

(*c*) *Four-day Rat* (*Plate* 3, *Fig.* 19)

Between 3 and 4 days, thickening and fusion of the periostea had continued, and there was extensive merging of the fibrous layers of the periostea lateral to the cartilage strips. The cartilage strips had increased in size in all directions,

but were still not in contact with one another, being separated by a dense concentration of unchondrified periosteal cells. The strip on the tibia was larger than that on the fibula.

The cartilage cells had enlarged and their cytoplasm often contained large vacuoles; they lay in a matrix resembling that present at 3 days. This matrix passed over on one side into bone by a swift but gradual transition and on the other into the joined fibrous layers of the periostea. The cartilage appeared first in the cambial layer of the periost, and then expanded in depth by extension of these changes into the fibrous layer (this is seen better in later stages) and in area by extension into the cambial layers of neighbouring regions.

(*d*) *Five-day Rat* (*Plate* 3, *Figs.* 20, 21)

In the material from the 5-day rat the cartilaginous character of the strips was more obvious and they were wider and thicker. Their increase in thickness occurred by further chondrification of the united fibrous periosteal layers, into which the hyaline matrix extended, while the cells enlarged and the previously dense nuclei became large and vesicular. There was still no cartilage in the angle on the medial aspect. Both transverse and longitudinal sections emphasize that the cartilage formed an alternative to bone for, with the increasing thickness of the bony wall in neighbouring regions, the cartilage occupied a depression in it, forming so much thickness of the wall of the bone as evidently would otherwise have been formed by bone (Plate 3, Fig. 17).

In the longitudinal series it is apparent that chondrification had not occurred through the whole area over which the periostea had fused, but only in a short proximal part, just distal to the point at which the shafts met. The histological picture suggests that the tibial and fibular surfaces did not lie flatly side by side, but that the fibula tried to push into the tibia (Plate 3, Fig. 20) by making a depression, or preventing the formation of bone, in a region which then filled with cartilage; and that a similar action of the tibia on the fibula was responsible for the formation of the fibular cartilage.

(*e*) *Six-day Rat* (*Plate* 3, *Figs.* 22, 23)

Chondrification had now extended across the fibrous periosteal layer which had hitherto separated the two cartilaginous strips, so that these now formed a single pad of cartilage connecting the two bones; the part of the cartilage derived from the fibrous layer nevertheless remained recognizable, for the cells retained their elongate form. Cartilage was also now present between the two bones where they came closest and in the angle between them on their medial aspects. In the 6-day animal the cartilage pad was about 1 mm long, the tibia and fibula about 10 mm long.

On the tibial side of the pad, resorption of the cartilage had begun from its base, and in the region of erosion capillary vessels and multinucleate cells which were presumably to be regarded as chondroclasts were present, coming from cavities of the underlying bone. In the transverse series the appearance of the sections suggested very strongly that the formation of cartilage might

be associated with injury to the bone as it is in fracture repair. In the tibia in particular the picture (Plate 3, Figs. 17, 23) in places suggested that part of its wall might at an earlier stage have been punched in by the fibula, and that new periosteal bone has later been developed on each side of the injury. It would not be surprising if the tibia and fibula, coming into contact in their distal regions because of their increasing girth, did thus inflict mechanical injury upon one another, and if cartilage were then to develop in association with the injury as it does in fractures, and in the region of micro-fractures at the proximal ends of tibiae and fibulae of guinea pigs recovering from scurvy (Murray and Kodicek 1949*c*). However, no incontrovertible evidence of this was obtained. Little or no evidence for such a view was found in the longitudinal series, but the supposed injury would be less easily recognizable in longitudinal sections; nor was evidence found in earlier or later stages.

(*f*) *Seven-*, 10-, *and* 12-*day Rats* (*Plate* 4, *Fig.* 25)

The cartilage pad is further enlarged in the 7-day specimens; at the same time resorption of the cartilage from below continues. At this time a shell of bone begins to form, deposited around the cartilage by enclosing periosteal cells. The result is the formation of a bridge of cartilage and bone joining the tibia and fibula; as the cartilage is destroyed and more and more bone replaces it, the bridge becomes purely bony, although vestiges of cartilage matrix can be found within it. The resorption of the cartilage, and the fact that the bony walls of the tibia and fibula beneath it were kept thin by the formation of cartilage instead of bone, make easy the establishment of a connection between the marrow cavities. Such a connection exists in the 12-day specimen (Plate 4, Fig. 25), in which bone now fills the angle which till recently existed on each side of the leg between tibia and fibula, so that the fused bones in this region acquire the rounded outline of a single bone.

V. The Tarso-Metatarsus of the Chick

Plate 4, Figs. 26-28

The fusion of the metatarsals has been studied in sections, mostly transverse, from chick embryos incubated for 7, 12, 14, 16, 17, 18, and 19 days.

In the 12-day material (Plate 4, Fig. 26), the three metatarsal shafts were still independent of each other, except that a plate of cartilage connected their proximal ends. Presumably this represents the distal tarsals, which in birds are fused with the metatarsals. A thin sheet of periosteal bone encircled each metatarsal, and bony trabeculae had appeared in the wide cambial zones.

Between the metatarsals a limit was placed on the width of the cambial zones, for where the fibrous layers of adjacent elements came into contact expansion of the cambial tissue was prevented.

Except on the apposed surfaces of adjacent metatarsals, the fibrous layers of the periostea were becoming thicker and more densely fibrous than they had been at 7 days. Further, they were joining up with one another, so that

a single fibrous layer was forming around the three shafts. On the apposed surfaces of adjacent metatarsals the fibrous layers were becoming exiguous, but each bone had here, as elsewhere on its surface, a thick or thin layer of cells which might have the appearance of osteoblasts.

The 14-day material still showed no sign of fusion of the metatarsal shafts. The bone was developing as a loose three-dimensional trabecular net, and from its structure it appeared that growth of the bone in thickness must be cyclic. Each cycle began with the development of trabeculae which in transverse sections radiated from the outermost circumferential lamella, and closed when the new circumferential lamella had been formed. Sections are readily found showing radiating trabeculae in process of formation, and others showing stages in the formation of circumferential lamellae. Nothing is certainly known of the factors which determine, at any particular area at any moment, whether radiating or circumferential bone shall be deposited.

The fusion of the shafts began, in this material, at 15 days (Plate 4, Fig. 27). Cartilage played no part. Between two apposed metatarsals, at first near their anterior and posterior surfaces, connecting trabeculae of bone formed, linking the two bones. This, of course, involved breaking across the two rows of osteoblasts and what little of a fibrous layer there was between the bones. Later, the circumferential lamellae between the opposed surfaces were similarly linked throughout their extent between anterior and posterior faces (Plate 4, Fig. 28). Meanwhile, the resorption of the cartilage shafts continued. At 19 days the only remaining indication of the triple origin of the bone which is seen in a transverse section near the middle of the shaft is a division of the marrow cavity into three by a couple of bony partitions: the surviving walls of metatarsals at adjacent surfaces (Plate 4, Fig. 28).

VI. The Tibio-Fibula and Radio-Ulna of the Frog

Plate 4, Figs. 29-32

The tibio-fibula was studied in a number of tadpoles and in a very young frog. In the tadpoles the youngest had hind limbs which, though small, were differentiated into their chief segments, while the oldest were advanced in metamorphosis, but still had tails.

In the youngest specimens (no external sign of anterior limbs) the tibia and fibula were still unconnected by hard tissue but shared a common periosteum which ensheathed both bones. In addition, the faces of the two bones turned towards each other were similarly covered, especially near their ends, each by its own periosteum, but towards the middle of the shafts these might be reduced to a single layer of osteoblasts. Beneath the periosteum each element had a thin periosteal bony covering; this was thicker on the sides of the bones facing away from one another than on the towards-facing sides. The periosteum was pressed fairly closely against the bones except where they faced one another. Here, the fibrous layer of each periosteum, instead of continuing around its own bone, passed across the angle separating the bones and became continuous with that of the other bone.

In the angles beneath it and between the bones was a loose tissue containing thin-walled blood vessels and cells resembling osteoblasts, evidently an enlargement of the periosteal cambium. The tendency to fusion was evident, for on the anterior and posterior aspects of each element the bony sheath tended to thicken, and to send plates which extended under the fibrous layer of the periosteum, across the angle between the bones, towards the other bone of the pair; but the plates from the two bones had not yet met. In the limb represented in Plate 4, Figure 29, these plates had joined and, besides the periosteal bone around each cartilaginous shaft, there is now a thin-walled cylinder of periosteal bone enclosing both shafts.

Later, bone formation beneath the common periosteum covering the angles on the anterior and posterior faces of the two bones extended over the whole diaphysis, and what had originally been two cartilaginous shafts, each later covered by a layer of periosteal bone, became a single bony shaft within which the original shafts were of course still easily recognizable (Plate 4, Fig. 30). At the same time the sheath of periosteal bone thickened greatly on the lateral aspect of the fibula and on the medial aspect of the tibia, and so did the bone bridging the angles between the cartilage models anteriorly and posteriorly, but that on the sides of the two elements facing each other thickened much less. Thus the two elements together appeared increasingly like a single bone. From the enlarged cambial zone between the shafts, blood vessels, accompanied by large uninucleate cells with foamy cytoplasm, invaded and began the resorption of the cartilage, while multinucleate osteoclasts were also present and appeared to be engaged in resorbing the thin layer of bone protecting the cartilage (Plate 4, Fig. 31). The final result (Plate 4, Fig. 32) of the resorption, as seen in a frog fixed 4 days after the disappearance of the tail, was a single shaft of hollow bone with a marrow cavity. This cavity was made up of the space provided by the resorption of the cartilage shafts, and of the space which lay between them.

The picture presented by the radio-ulna differed from this only in minor details; it is evident that these two bones fuse in the same manner as do the tibia and fibula.

VII. Discussion

Possibly the most interesting result which emerges from this study is the intermediary formation of cartilage in the normal fusion of the rat tibia and fibula and in the abnormal or sporadic fusion in the guinea pig, for one would expect fusion of bones to occur as it does in the metatarsals of the chick and in the frog's tibia and fibula.

It is of course well known that the periosteum and endosteum are both on occasion capable of forming cartilage in tissue culture (Fell 1932, 1933), as in most fracture calluses, and there has been much controversy about the importance of mechanical and other factors in the induction of chondrification. It seems clear enough that formation of cartilage in the embryo and in periosteum or endosteum *in vitro*, occurs independently of movement and pressure between parts.

What evidence we have of ectopic and even extra-skeletal cartilage formation suggests humoral, rather than mechanical, factors as the causal agent. Thus, in many of Levander's (1938) experiments, intramuscular cartilage was formed after injection of alcoholic extracts of bone, or even of alcohol alone (Annersten 1940), results which it is much easier to attribute to humoral than to mechanical influences. One must conclude that cartilage formation often occurs in response to other stimulation than special mechanical conditions.

Nevertheless, it seems clear that, if certain ill-defined mechanical conditions exist, cartilage can be formed by cells of the skeletal group which do not normally chondrify. These conditions seem to involve pressure and very probably the shearing of layers of the tissue upon one another (Roux 1895); circumstances which exist in fracture calluses and between the fusing tibiae and fibulae of guinea pigs and rats.

The histological picture (e.g. Plate 1, Fig. 4) seems strongly to favour the view that the pressure across a fracture is taken up by cartilage before the development of the bony callus is complete, and Krompecher (1937) found in dogs that the formation of cartilage in the callus could be prevented by placing the fracture region under tension instead of under pressure. Glücksmann's (1939) *in vitro* studies amount to an experimental demonstration of cartilage formation in the periosteum subjected to a combination of pressure and shear, and similar evidence is provided by the new cartilage covering the opposed surfaces in nearthroses. The formation of cartilage in the rat and guinea pig when the tibiae and fibulae come into contact is at least in harmony with the ideas of Roux (1895) and Glücksmann (1938, 1939). Some of the longitudinal sections suggest that in the rat each bone does in fact subject the periosteum of the other to both forms of strain. It should be mentioned that Glücksmann's evidence shows that in his material simple pressure was a sufficient stimulus.

Certain authors, such as Koch (1924), have contended that chondrification in fracture calluses is not mechanically induced, and see the determining factor in the poor vascularization of parts of the soft callus; in such poorly vascularized regions are formed dense masses of cells which chondrify. It is supposed that some factor associated with this tissue structure is the determinant of chondrification; of course, as is realized by two recent protagonists of this view, Pritchard and Ruzicka (1950), poor vascularity and cellular density may themselves be results of local mechanical conditions.

Neither cellular density nor poverty of vascularization can easily be made responsible for the periosteal chondrifications here described, at least in the rat, for here the cambial layer begins its chondrification before any dense mass of cells is formed, indeed without any increase in density by comparison with the cambial layer of other parts of the periost. It therefore seems easier to regard the formation of dense cell masses rather as part of the chondrification process than as its determining factor, and to seek for this factor among the special biochemical conditions which are set up by some but not by all sorts of skeletal injury (for example, at fractures under pressure but not at fractures under tension, and not at amputation stumps). Such a view is in harmony with the tissue culture results, in which of course the formation of bone or cartilage

does not depend on vascularization, which is always absent. It is also in harmony with the appearance of mutual injury by the tibia and fibula of the rat.

It seems possible to conclude:

(1) That presumptive cartilage cells of the embryo chondrify independently of mechanical stimulation, the determining agent being presumably humoral;

(2) That cells which do not normally chondrify, but which belong to the skeletal group, form cartilage if they are subjected to certain mechanical conditions, and that these conditions include pressure and probably shear;

(3) That cells of the non-skeletal connective tissue group can form cartilage only if they undergo an induction, presumably by a humoral agent, and that no further mechanical stimulation seems then to be required.

A question closely connected with that of cartilage formation is the possible distinction, in respect of powers of differentiation, between the fibrous and cambial layers of the periosteum. The structural difference is obvious and implies a physiological distinction in ability or readiness to form collagen fibres. That other distinctions exist has been shown by Fell (1932) who found that, while ossification occurs in the periosteum from young embryos isolated *in vitro*, it does not do so if derived from older embryos or hatched chicks, although bone and cartilage may develop from the endosteum of such chicks. In other words, the medium she used was adequate for bone and cartilage formation by cells capable of these forms of differentiation, but the fibroblastic layer of the periosteum nevertheless failed to form either of these tissues. Wurmbach (1928), in a study of fracture repairs in mice, found that the undamaged fibrous layer appeared incapable of chondrification, but that torn pieces of it may do so with difficulty. It is thus of interest that in the tibio-fibular fusions of both guinea pigs and rats, chondrification, while it started in the cambial tissue, spread across the fused fibrous layers, and that these chondrified, together with any non-periosteal connective tissue which there may have been between them. This was not merely a passive engulfment of fibroblasts by cartilage matrix emanating from the cambial layer, but involved enlargement and vacuolation of the cells. In the guinea pig the structure of the older cartilage pads (Plate 2, Figs. 14, 15) left little doubt of the transformation of the fibrous layers into cartilage; the only alternative would be that the cells and fibres of the fibrous layer had died and disappeared, and of this no sign was found. In the fusing tibia and fibula of the rat the originally fibrous layers were recognizable, even after the hyalinization of the matrix, from the greater flatness of the cells, but these too became vacuolated though perhaps never as much so as the cells derived from the cambial layers.

It is impossible to point to any histological reason for the differences in the fusion process between the rat and the guinea pig on the one hand, and the chick and tadpole on the other. That the periostea are capable of cartilage formation is shown in both birds and frogs by their behaviour in fracture repair (Roggemann 1930; Pritchard and Ruzicka 1950). Possibly an explanation might

be found in the occurrence of relatively less movement between the fusing bones in the chick and tadpole; but this would be very difficult to demonstrate, even if it were true. Still a third mode of fusion was reported by Harris (1936), who found that in the sheep embryo the second and fifth metacarpals become buried in the cartilage of the much larger third and fourth.

VIII. Acknowledgments

Part of the expenses of this work was met by the Medical Research Council of Great Britain; and part from the Commonwealth of Australia Research Grants Fund; thanks are tendered to both these bodies. I wish also to thank Mrs. Margaret Spencer, lately of this Department, who made the first study of the fusing tibia and fibula in the rat, my research assistant, Miss Isobel Bennett, especially for the preparation of many series of sections, and Mr. L. Congdon, for assistance with the photographs.

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Explanation of Plates 1-4

Abbreviations used in the plates: e. cart., early cartilage developing in the periosteum; Fib., fibula; Fib. bone, bone of the fibula (before thickening); Fib. th., periosteal bony thickening of the fibula; fibr. per., fibrous periostea; Per. and cart., periostea (fused) with cartilage; Tib., tibia; Tib. bone, bone of the tibia (before thickening).

The magnifications given are those at the eyepieces, the changes in "empty magnification" caused by later enlargement and reduction being disregarded.

Plate 1

Fig. 1.—Guinea pig; the fibula 1 day after fracture. × 25.

Fig. 2.—Guinea pig; the fibula 7 days after fracture. × 25.

Fig. 3.—Guinea pig; part of the callus 9 days after the fracture of the fibula; young cartilage with transition into fibrous connective tissue (below) and granular bone (left). × 100.

Fig. 4.—Guinea pig; the fibula 11 days after fracture; two masses of cartilage intervene between the trabeculae of the bony callus. × 20.

Fig. 5.—Guinea pig; young cartilage cells in the callus 9 days after fracture of the fibula. × 400.

Fig. 6.—Guinea pig; callus cartilage 11 days after fracture of the fibula with transitions to connective tissue (right and left) and to granular bone (above and below). × 80.

Fig. 7.—Guinea pig; callus cartilage 16 days after fracture of the fibula; capsules and matrix well developed but little or no vacuolation. × 425.

Fig. 8.—Guinea pig; hypertrophied and vacuolated cells in the callus cartilage 13 days after fracture of the fibula. × 1000.

Fig. 9.—Guinea pig; transition from cartilage into bone, in the callus 14 days after fracture of the fibula. × 340.

Plate 2

Fig. 10.—Guinea pig; chondroclasts (above) apparently resorbing cartilage; below, to right and left, bone. From the callus 12 days after fracture of the fibula. × 400.

Fig. 11.—Guinea pig; longitudinal section through early cartilage pad, 1 day after fracture of the fibula. × 80.

Fig. 12.—Cartilage pads on the apposed surfaces of the right tibia and fibula in a normal guinea pig whose left fibula had been fractured 94 days before death. The periostea have chondrified (thionin-positive) but the cartilage pads have not fused but instead seem to have formed a nearthrosis. × 80.

Fig. 13.—Guinea pig; 35 days after fracture of the fibula. A cartilage pad has formed in the fused periostea between the diaphyseal thickenings (animal partially deficient in vitamin C, belonging to Expt. 5, group 4, of Murray and Kodicek 1949*a*). × 37·5.

Fig. 14.—Guinea pig; 85 days after operation. Cartilage pad between tibia (below) and fibula (above); notice that at each end of the cartilage it continues into unaltered periostea. Animal partially deficient in vitamin C, belong to Expt. 2, group 1, of Murray and Kodicek (1949*a*). × 25.

Fig. 15.—Part of the cartilage pad from the other leg of the same guinea pig as Figure 14 (in this instance both legs were operated). Notice that resorption of the pad is beginning on each side, from cavities in the bone. × 32·5.

Fig. 16.—Guinea pig; 94 days after fracture of the fibula; the tibia and fibula are joined by a bony bridge. × 20.

Plate 3

Fig. 17.—Six-day rat. T.S. tibia and fibula, showing the cartilaginous pad by which they are joined. × 30.

Fig. 18.—One-day rat. Part of L.S. tibia and fibula, showing the fused tibial and fibular periostea, near the distal ends. × 30.

Fig. 19.—Four-day rat. Part of T.S. tibia and fibula, the fibula above, the tibia below. Early chondrification in the cambial zones of the tibial and fibular periostea. × 120.

Fig. 20.—Five-day rat. Part of L.S. tibia and fibula. Tibia above, fibula lower left, distal end to the right. Cartilage pads have formed in both periostea in the region of impingement of the fibula on the tibia. × 60.

Fig. 21.—Five-day rat. Part of T.S. tibia and fibula, the fibula above. Cartilage has formed in the cambial layers of the two periostea and there is early chondrification of the fused fibrous layers. × 120.

Fig. 22.—Six-day rat. Part of T.S. of tibia and fibula, fibula above. Chondrification of the intervening fibrous layers of the periostea has united the originally separate cartilage pads. Resorption of the cartilage is seen in progress on the tibial side. × 100.

Fig. 23.—Six-day rat. Part of T.S. tibia and fibula, showing a histological picture suggesting that the cartilage formation may be a reaction to mechanical injury mutually inflicted by the two bones. See also Figure 17. × 80.

Fig. 24.—Seven-day rat. Part of T.S. tibia and fibula, the fibula above, showing an advanced cartilage pad. × 100.

Plate 4

Fig. 25.—Twelve-day rat. T.S. tibia (below) and fibula (above), showing the bony junction and the continuity of the two marrow cavities. × 120.

Fig. 26.—Twelve-day chick embryo. T.S. metatarsals 2-4. × 37·5.

Fig. 27.—Fifteen-day chick embryo. Part of T.S. of the metatarsals; on the left the two metatarsals are connected by a narrow trabecula of bone. × 80.

Fig. 28.—Nineteen-day chick embryo. T.S. tarso-metatarsus. Metatarsals 2-4 are firmly united, but their separate origin is shown by the long trabeculae in the position of the original adjacent walls. × 30.

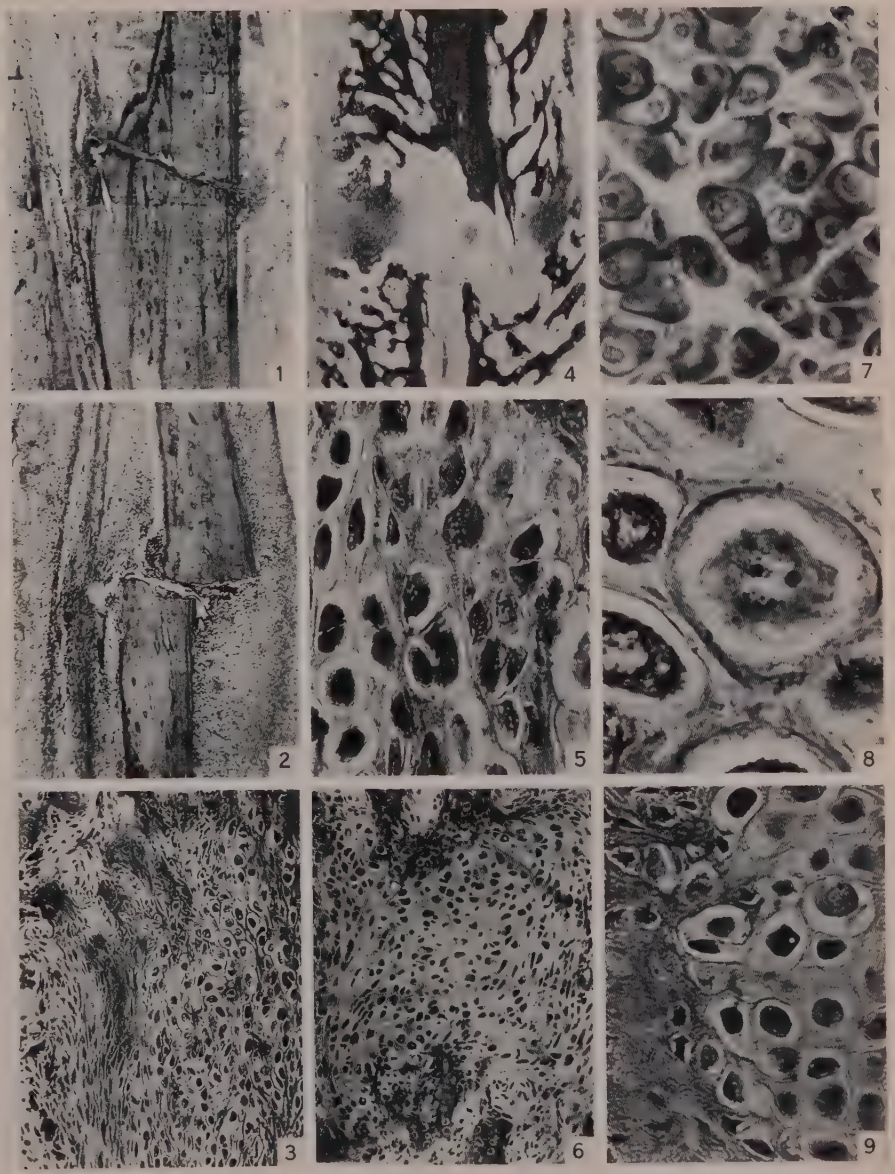
Fig. 29.—Tadpole with posterior limbs, but anterior limbs not yet visible. Each cartilaginous shaft has a sheath of periosteal bone, and a cylinder of periosteal bone encloses both shafts. × 120.

Fig. 30.—Tadpole with fore limbs not yet emerged, but visible through the operculum. T.S. tibia and fibula. × 120.

Fig. 31.—Tadpole with fore limbs not yet emerged, but visible through the operculum. T.S. tibia and fibula, showing the formation of the common marrow cavity. × 120.

Fig. 32.—Frog, 4 days after completion of the resorption of the tail. T.S. tibio-fibula. × 120.

BONE FUSION AND SECONDARY CARTILAGE FORMATION



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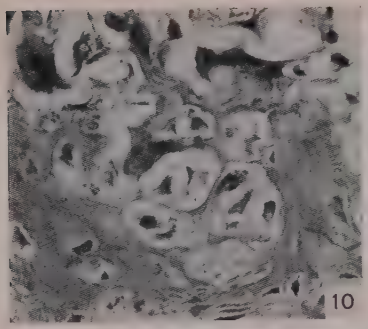

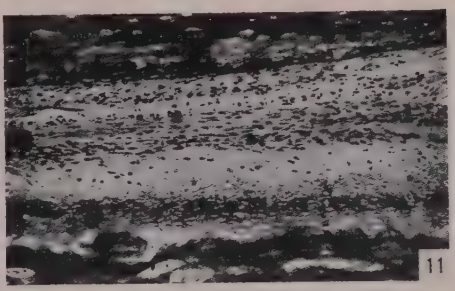

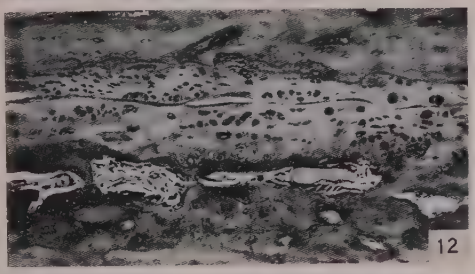

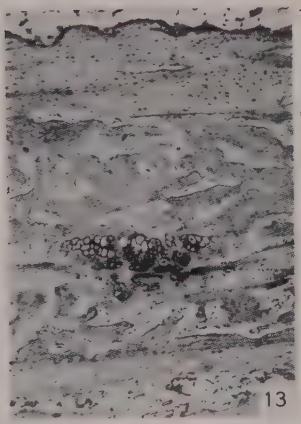

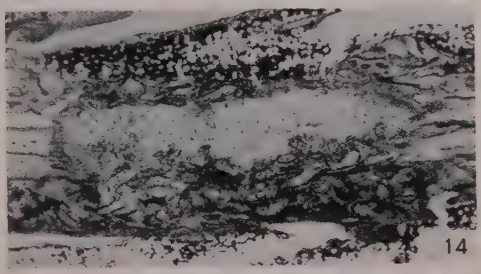

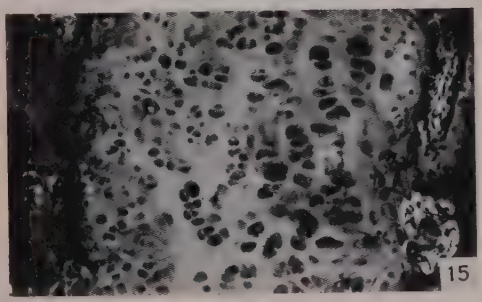

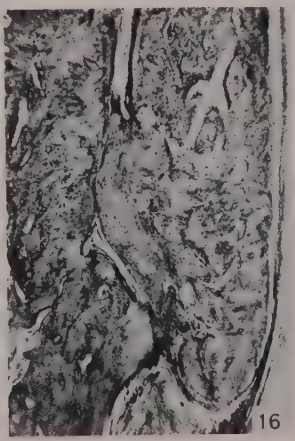

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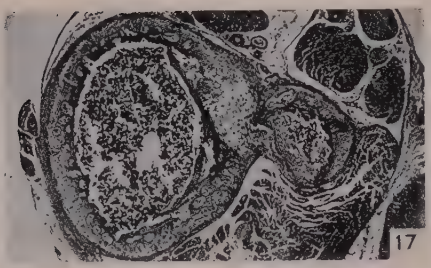
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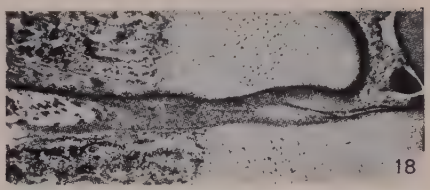
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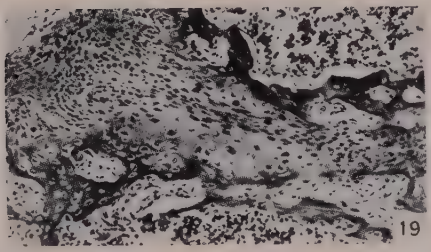
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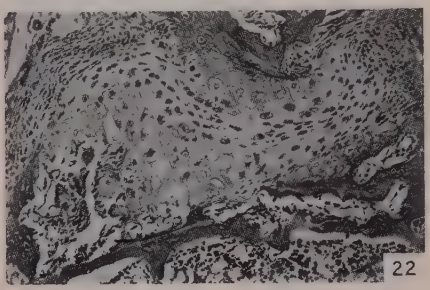
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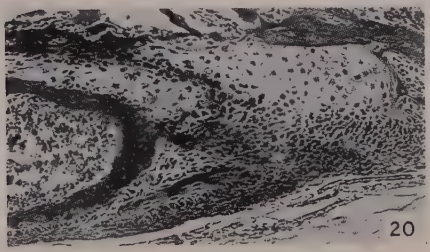
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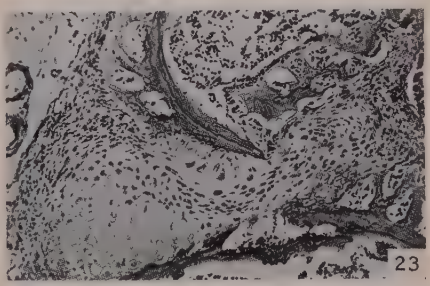
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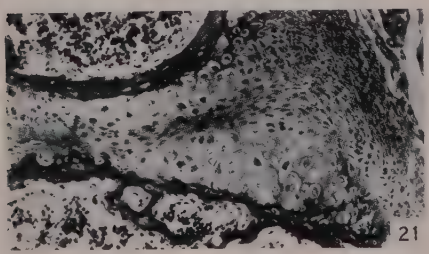
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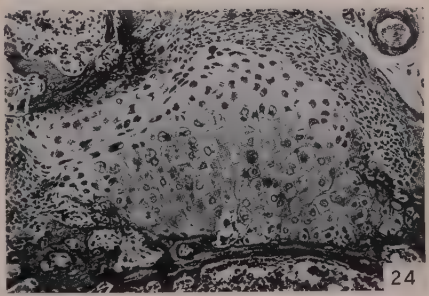
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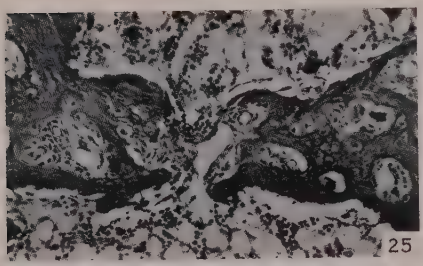

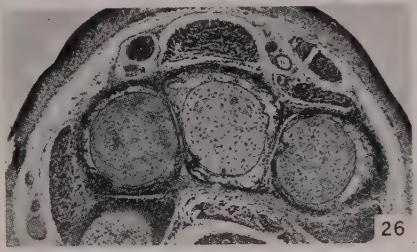

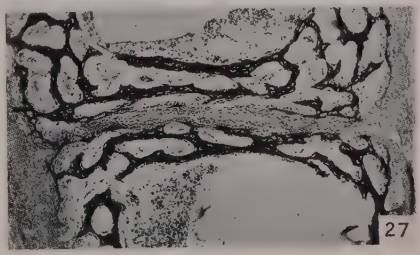

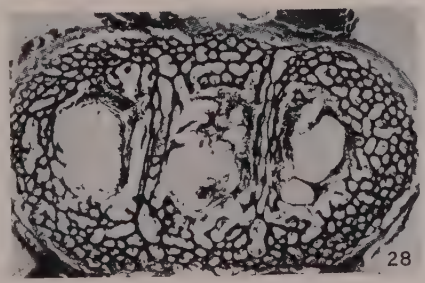

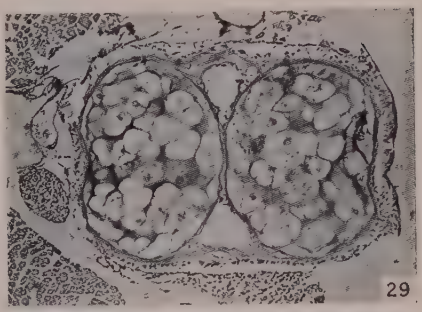

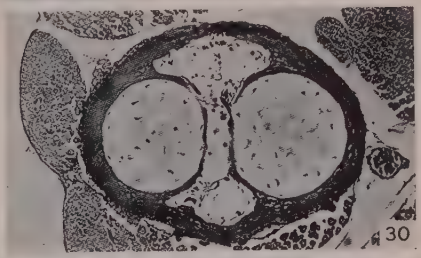

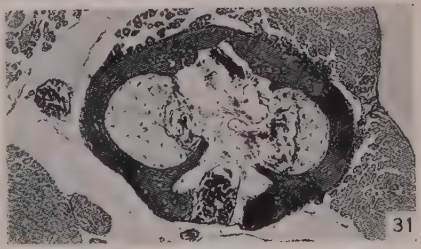

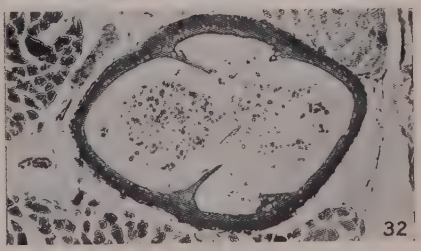

STUDIES IN FUNCTIONAL AND ANALYTICAL CRANIOLOGY

I. THE ELEMENTS OF ANALYSIS

By R. TUCKER*

[*Manuscript received May* 31, 1954]

Summary

The method of analytical investigations of functions connected with the taking of food is described. The distribution of stresses within the cranium and their simple relations; the circumscribed and dispersed stresses, together with the origin of transmitted and shearing stresses; the role of moment of force in masticatory movements; and the factors influencing the arising of forces, are also discussed.

I. SCOPE OF THE INVESTIGATIONS

Among many functions of the mammalian skull, the intake of food and its mastication are two of the most important. Moreover, that function involves stresses of considerable magnitude. This means mechanically that they should have a morphogenetic and functional significance. They can also open the way into a completely unknown field. Consequently in the series of papers on the functional and analytical craniology, of which this is the first, the skulls are studied in regard to function connected with the taking of food and its preparation for swallowing.

II. STRAINS AND STRESSES

Throughout these papers the terms "strain" and "stress" are used in accordance with the definitions given by Lemon and Ference (1946, p. 108) which set out that stresses are deforming forces. Such forces distributed over areas are called "surface forces" and are to be contrasted with the body forces (the weight of an object, and so on). By definition:

$$\text{Stress} = \frac{\text{force that balances the applied force}}{\text{area over which the force acts}} = \frac{F}{A} .$$

When the structure is changed in shape the deformations are called "strains." Accordingly the law of Hook may be stated as

$$\frac{\text{stress}}{\text{strain}} = \text{constant} — \text{modulus of elasticity.}$$

III. THE BASIC ELEMENTS

The simplest structural elements which are present in all mammalian skulls and simultaneously form the functional frame for the taking of food are:

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(*a*) Two elastic bands representing the temporal muscle (Fig. 1, *a*) and the masseter (Fig. 1, *b*); and

(*b*) Two relatively inelastic bony bars (the living bone has an organic part as well), namely, the mandible (Fig. 1, *c*) and maxilla (Fig. 1, *d*).

The latter elements (*b*) are connected by means of a cranio-mandibular joint (Fig. 1, *e*).

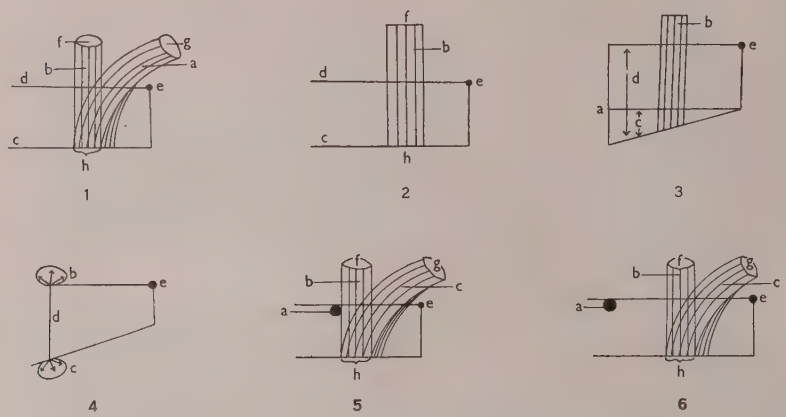

Fig. 1.—Basic elements: *a*, temporal muscle; *b*, masseter; *c*, mandible; *d*, maxilla; *e*, cranio-mandibular joint; *f*, *g*, *h*, insertions of masseter and temporal muscle.

Fig. 2.—Diagram of contracted masseter. *b*, Masseter; *c*, mandible; *d*, maxilla; *e*, cranio-mandibular joint; *f*, *h*, muscular insertions.

Fig. 3.—Diagram of the action of masseter during mastication. *a*, The position of mandible corresponding to those in Figure 2; *b*, masseter; *c*, degree of elongation of masseter; *d*, the obstacle; *e*, cranio-mandibular joint.

Fig. 4.—Diagram illustrating the relations between the position and stresses. *b*, Stresses in maxilla; *c*, stresses in mandible; *d*, the obstacle (food); *e*, cranio-mandibular joint.

Fig. 5.—Location of the circumscribed stresses in the region of masseter. *a*, Location of stresses (position) in the fourth premolar; *b*, masseter; *c*, temporal muscle; *e*, cranio-mandibular joint; *f*, *g*, *h*, insertions of masseter and temporal muscle.

Fig. 6.—Location of the circumscribed stresses in the premaxilla. *a*, Location of stresses (position); *b*, masseter; *c*, temporal muscle; *e*, cranio-mandibular joint; *f*, *g*, *h*, insertions of masseter and temporal muscle.

It is easy to see that the very first stress set up by the contraction of the masseter and temporal muscles appears at their attachments (Fig. 1, *f*, *g*, *h*) while a secondary stress is exerted in the glenoid cavity (Fig. 1, *e*).

This simple and diagrammatic arrangement of stresses (Fig. 1) is true for all non-masticatory states. In other words, when the maxillo-mandibular contact is neither changed nor complicated by the accumulation of food or other hard material between the mandible and maxilla, and when, consequently, the accessory structures designed for fighting the tertiary stresses are not developed or functioning.

The tertiary stresses are developed in the upper and lower jaw-bones where accumulations of food lie between them.

IV. Mastication

Mastication introduces a new mechanical factor which, functionally, opposes the action of the contracting muscles and keeps the upper and lower arcades of teeth apart. Figure 2 demonstrates the contracted masseter, while Figure 3 shows the masseter not fully contracted and hampered in its contraction as long as the block '*d*' exists (Fig. 3, *d*). The arrow '*c*' demonstrates the degree of elongation of the masseter caused by '*d*', which in contracting exerts a force which sets up its share of the primary, secondary, and tertiary stresses.

On the other hand, this elongation depends on the size and position of the obstacle '*d*' (Fig. 3). Accordingly, the corresponding force is related to the size and position of the obstacle '*d*' (Fig. 3). It can also be demonstrated briefly in the following way:

$$F \; r \; e$$
$$e \; r \; SP$$
$$F \; r \; SP$$

when r is the symbol of relation; e the degree of muscular elongation (extension); S the size and P the position of the obstacle; F the force.

V. Position

For the sake of simplicity in the introductory analysis, SP can be reduced to its position P only. By assuming that the size S is in all cases constant, we obtain the obstacle '*d*' (Fig. 3) characterized by one property only; consequently if S is constant the relation $F \; r \; SP$ can be written as $F \; r \; P$.

It would be a hopeless and unnecessary task to analyse all positions of a given obstacle (or particle) in relation to the forces in the masseter and temporal muscles and to the primary, secondary, and tertiary stresses exerted by them. For the purpose of the functional analysis of the cranial structure it is sufficient to investigate the positions which are common enough and of particles of considerable size which can affect the mechanics of the whole skull.

Therefore in these papers the position (P) will be taken as the site at which the stresses set up in mastication are those great enough, compared with the sectioned area of the particular cranial structure, to determine the adaptive character of the bony structure.

VI. Positions and Stresses

At this stage the relations as demonstrated by Figure 3 can be extended to the effects of both ends of the obstacle '*d*' (Fig. 3, *d*) onto the corresponding parts of the mandible and maxilla. The resistance of food (in the given position) against the force which originates from masticatory muscles creates stresses which radiate into the surrounding bone (Fig. 4, *b*, *c*). Consequently stresses appear in defined areas of the mandible and maxilla. Those areas

betrayed, in the decalcified skulls, the existence of stresses by detectable structural transformations. Hence the defining of position, by the stresses and morphological character depending on this, is possible and justified. Moreover, by the analysis of stresses in the organic structures the position can be defined with a sufficient degree of accuracy.

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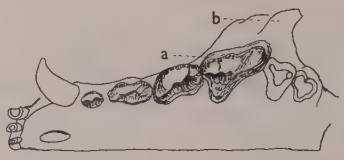

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Fig. 7.—Area of circumscribed stresses in *Nimravus gomphodus* Cope (from Abel after Matthew). p^4, Fourth premolar; *Z*, anterior end of the zygomatic arch.

Fig. 8.—Dentition of *Ictitherium robustum* (redrawn from Nicholson after Gaudry). *a*, Carnassial; *b*, zygomatic arch.

Fig. 9.—Teeth and the alveolar process in the region of the anterior end of the zygomatic arch in *Hyena spalaea* (redrawn after Nicholson). *a*, Zygomatic arch.

Fig. 10.—Premaxillary area of circumscribed stresses in *Tillotherium fodiens* (redrawn after Nicholson). *a*, Premaxilla; *b*, incisors.

VII. Kind of Stresses

The survey of the topographic distribution of the main masticatory stresses in a variety of mammalian skulls shows that the stresses are either (*a*) circumscribed, or (*b*) dispersed. Stresses of each kind create the specific static and mechanical dispositions of the cranial structure. At the level of the alveolar processes of the maxilla and mandible disposition is demonstrated by the differentiation in size and form of the incisors, canines, premolars, and molars; in the height of mandible and maxilla; in the straight or curved outline of the alveolar processes; in the presence or absence of the toothless margin (margo adentalis), and so on.

VIII. The Circumscribed Stresses

The stresses I am describing as circumscribed are limited to a relatively small area, but are of great magnitude. The magnitude is a function of the limited area they act upon.

In the mammalian skull, they have the following easily demonstrated locations: (*a*) In the region of the anterior end of the zygomatic arch, most often at the position of the fourth premolar (as in carnivores); (*b*) In the area of premaxilla, most often in incisors (rodents) and in some species also in the canine; (*c*) In the glenoid area.

Fig. 11.—Premaxillary area of circumscribed stresses in *Oryctolagus cuniculus*. *a*, Incisors; *b*, premaxillary.

It is important, as will be shown later, that in the first and third cases the circumscribed stresses are situated in the region of the masseter (Fig. 5, *a*) while in the second, in the region lacking masticatory muscles (Fig. 6, *a*).

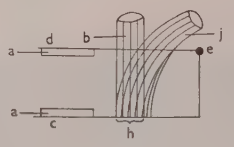
The development of structures corresponding to the circumscribed stresses in the area of the anterior end of the zygomatic arch is demonstrated in Figures 7, 8, and 9. Figure 7 shows the maxillary alveolar process of *Nimravus gomphodus* Cope (Felinae) with the characteristic development of the fourth premolar under the anterior end of the zygomatic arch. For relation of this point with the masseter, compare with Figure 5. Similar examples are given by the skulls of *Ictitherium robustum* (Viverinae) (development of the carnassial) (Fig. 8) and *Hyena spalaea* (Hyenidae) (Fig. 9).

The circumscribed stresses in the region of premaxilla and the corresponding structural characteristics may be observed in the skull of *Tillotherium fodiens* (Tillodontia) (Fig. 10) and a rabbit (Fig. 11).

IX. The Dispersed Stresses

In skulls subjected to dispersed tertiary stresses the contacting surfaces of maxilla and mandible are markedly enlarged. This results from: (*a*) Enlargement of occlusal surface of one or two teeth (Fig. 12); or (*b*) Formation of a row of teeth fairly uniform in their size and height (Fig. 13).

The first case is well demonstrated by the teeth of the elephant (Proboscidea) (Figs. 14 and 15) and in a different form by *Hypsiprymnus cuniculus* (Marsupialia) (Fig. 16) as well as by *Plagiaulax* (Multituberculata) (Figs. 17 and 18).



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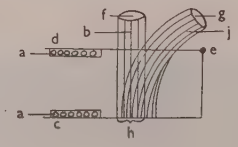

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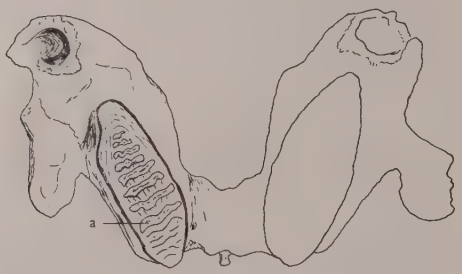

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Fig. 12.—Diagram of morphological bases for dispersed stresses. *a*, Enlargement of the occlusial surface of a tooth; *b*, masseter; *c*, mandible; *d*, maxilla; *e*, cranio-mandibular joint; *f*, *g*, *h*, insertions of masseter and temporal muscle; *j*, temporal muscle.

Fig. 13.—Diagram of morphological bases for dispersed stresses. *a*, A row of uniformly shaped teeth; *b*, masseter; *c*, mandible; *d*, maxilla; *e*, cranio-mandibular joint; *f*, *g*, *h*, insertions of masseter and temporal muscle; *j*, temporal muscle.

Fig. 14.—Mandible and occlusional surface of a tooth of *Elephas meridionalis* (redrawn after Adams). *a*, The tooth.

In the second case the teeth may be pressed each against the other, forming a continuous structure, as in ruminants or horse, or their crowns form a serrated structure, as in many Mesozoic mammals. Both variants are well developed in a great number of mammals. Figure 19 demonstrates the formation of segmented (many teeth) contacting surface in *Macropus benetti* (Marsupialia) while Figure 20, that in fossil man, *Homo heidelbergensis* (*Europanthropus*

heidelbergensis). The serrate outline of uniform teeth is demonstrated in *Amphitherium* (Fig. 21).

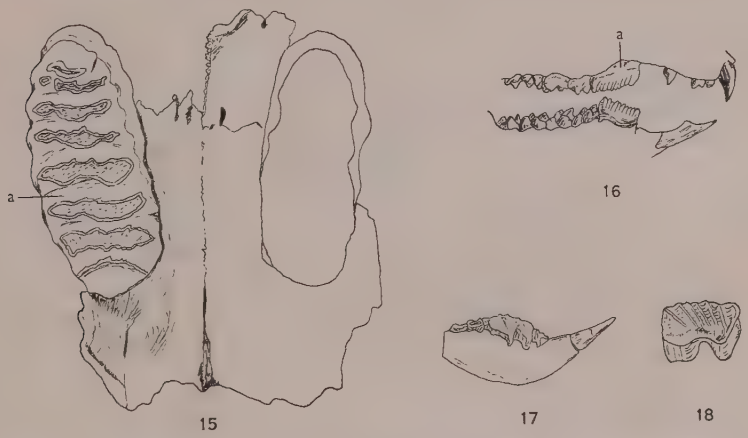

Fig. 15.—Occlusional surface of the molar in the maxilla of *Elephas meridionalis* (redrawn after Adams). *a*, Third milk molar.

Fig. 16.—Elongation of the first premolar of *Hypsiprymnus cuniculus* (redrawn after Nicholson). *a*, First premolar.

Fig. 17.—Enlargement of premolars in *Plagiaulax minor* (redrawn from Nicholson after Owen).

Fig. 18.—Enlarged premolar of *Plagiaulax becklesi* (redrawn from Nicholson after Owen).

X. Shearing and Transmitted Stresses

The most important consequence of the circumscribed and dispersed stresses is the kind of stresses raised by them within the cranial structures. Mechanically, maxilla and mandible are beams (*a*) placed on the broad supporting structure when the contacting surface is large, or (*b*) supported in distant points which are far apart from each other, but are joined by powerful connective structures.

In case (*a*) when mandible and maxilla are pressing each other along a continuous surface of contact, shearing stresses arise (Fig. 22). In skulls developed along these lines the shearing stresses are the only stresses which can destroy the structure. They are great enough to affect the development of structure. What is more, they are local stresses and they are resisted at the very place, or close to the place, of their origin.

It is different in case (*b*) which contains the skulls with circumscribed stresses. The area is too small, the stresses too great to be resisted locally. They are resisted not only by the structures they directly act upon. They are transmitted sometimes to the distant points of the skull. Accordingly, various structures develop for the transmission of these stresses. More will be said

about this later. Now it is only important that the cranial beam becomes supported at distant points; the mechanical stage is set up for the arising of moments of force (Fig. 23, *A*, *B*).

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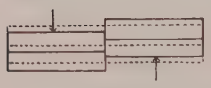

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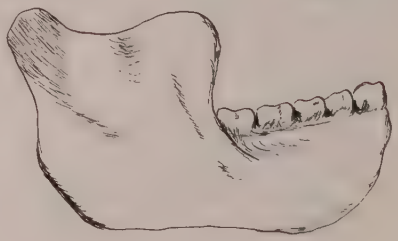

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Fig. 19.—Formation of the segmented contacting surface in *Macropus benetti*.

Fig. 20.—Formation of segmented contacting surface in man from Mauer (redrawn from Wust after Weinert).

Fig. 21.—Serrate outline of the lower row of dentition in *Amphitherium* (redrawn after Nicholson).

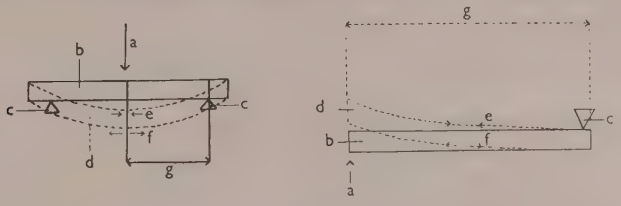
Fig. 22.—The origin and action of shearing stresses.

XI. Musculo-mandibular Relations

The moment of force has also another application in the function of mastication. During the mandibular movement illustrated in Figure 3, the points situated on the alveolar process move more quickly when they are further from the muscular insertions, but the force exerted at them is smaller than at points located nearer to the masseter. For points in the alveolar process near the masseter the distance is less but the force greater. The length of the bony bar increases the speed, but decreases the force.

Most of the fibres of the temporal muscle are situated much more posteriorly than those of the masseter. The typical location for the insertions of temporal muscles is the temporal (coronoid) process of the mandible which makes the bony bar moved by the temporal muscles much longer than that in the contraction of the masseter. The advantages and disadvantages of both masticatory muscles relative to their topography are demonstrated in Figure 24. It is easy to see that only the masseter is a "crushing" muscle and that the lever formed by its insertions, especially in comparison with the temporal

muscle, is one of sustained power. The importance of the temporal muscle is emphasized in its ability to initiate the speedy mandibular movement.



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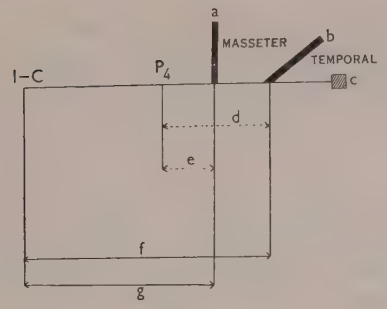

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Fig. 23 *A, B.*—General distribution of stresses in the supported beams and the arising of the moment of force. (The static conditions of the brevіarcuate and longoarcuate skulls. The maxillary and mandibular beams are resting only on certain points. It illustrates the tendency to bend, transmission of stresses, and development of torque.) *a,* The force; *b,* the beam; *c,* supporting points; *d,* the deformation of beam; *e,* compression forces; *f,* tension forces; *g,* lever arm.

Fig. 24.—The primary differences in moment during contraction of the masseter and temporal muscle. *a,* Masseter; *b,* temporal muscle; *c,* mandibular joint; *I-C,* incisors and canines; P_4, carnassial; *d,* lever arm of temporal muscle when position of force is at P_4; *e,* lever arm of masseter when position of force is at P_4; *f,* lever arm of temporal muscle when position of force is at incisors; *g,* lever arm of masseter when position of force is at incisors. To avoid unnecessary complication of diagram only incisors and premolar 4 are indicated but similar analysis could be made for any other point in the alveolar process, for instance P_1, P_2, P_3, M_1, M_2.

XII. The Force

Accordingly, the relations of basic elements to each other as demonstrated in Figure 1 were over-simplified. The force is not only characterized by the position and its after-effects, but also by the topography of both masticatory muscles. Also the elongation of muscle (Fig. 3, *c*) is not a simple function of the position of food but also of the position of the insertions of the muscle and these in turn are conditioned by many factors, one of them being the length of the skull (for the upper insertions of temporal muscle). The moments of both masticatory muscles are primarily different but these increase or decrease

as the position of food is farther from or nearer to the musculature, respectively.

Therefore the relations $F\ r\ P$ (Section V) can now be written $F\ r\ PP^1$, when P^1 is the location of the muscular insertion. Because for both muscles

$$P^1 = Pc + Pm,$$

when c is the cranial and m the mandibular insertion, then

$$P^1 = TPc\ MPc\ TPm\ MPm,$$

(M being masseter and T temporal muscle) and

$$F\ r\ PTPc\ MPc\ TPm\ MPm.$$

The correlations of all these elements give a number of variations in stresses which influence the structure of the skull.

XIII. Bones and Stresses

All living structures respond to a variety of stimuli. Mechanical stimulus is one of the most common and most important. The behaviour of long bones submitted to stresses has been investigated by a number of workers. The trajectory theory was one attempt to explain the structure along mechanical lines. A critical review of these investigations was published by Murray (1936). However, in this matter nothing is known about the bones of the skull. On the other hand, even small changes in the location of secondary and tertiary stresses seem to have distant repercussions (Tucker 1953*a*, 1953*b*). Apparently the morphofunctional connections (Tucker 1953*c*) are very strong. The detailed investigations of the cranial relations based on the kind of stresses described above will be carried on in subsequent papers.

XIV. Acknowledgment

I am greatly indebted to Professor P. D. F. Murray, University of Sydney, for critically reading the manuscripts of Parts I-VI and offering many helpful suggestions; he also suggested the subject of Part V.

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STUDIES IN FUNCTIONAL AND ANALYTICAL CRANIOLOGY

II. THE FUNCTIONAL CLASSIFICATION OF MAMMALIAN SKULLS

By R. Tucker†

[*Manuscript received May* 31, 1954]

Summary

The stresses exerted in the alveolar process are transmitted onto other parts of the skull by means of arches or resisted locally. The arches are: (*a*) long, (*b*) short, and (*c*) flat. Accordingly, the functional taxonomy of the mammalian skull is based on the conception of the breviarcuate skull (short arch), the longoarcuate skull (long arch), and the planoarcuate skull (flat arch). The structural evolution of these types from the primitive skull is illustrated and described.

I. Material Studied

Functionally, one of the most important factors in skull structure is the concentration or diffusion of stresses in the various regions (Tucker 1954) and this has been studied in a wide range of species as follows:

Reptiles:

*Placodus** (Placodontidae, Placodontia, Sauropterygia); *Edmontosaurus** (Hadrosauridae, Ornithopoda, Ornitischia); *Delphinognatus** (Tapinocephalidae, Tapinocephalia, Therapsida); *Dimetrodon** (Sphenacodontidae, Sphenacodontia, Pelycosauria); *Cynognathus** (Cynognatidae, Cynodontia, Therapsida); *Dromatherium sylvestre** (Dromatheridae, Ictidosauria).

Mammals:

Ornithorhynchus anatinus (Ornithorhynchidae, Monotremata, Prototheria); *Echidna aculeata typica, E.a. multiaculeata* (Echididae, Monotremata, Prototheria); *Amphilestes broderipi,** *Priacodon,** *Triconodon,** *Cynognathus** (Triconodontidae, Triconodonta, Theria); *Spalacotherium** (Spalacotheridae, Symmetrodonta, Theria); *Plagiaulax beclesi,** *P. minor* (Plagiaulacidae, Multituberculata, Allotheria); *Taeniolabis* (Taeniolabidae, Multituberculata, Allotheria); *Amphitherium** (Amphitheridae, Trituberculata, Pantotheria); *Phacolestes** (Dryolestidae, Trituberculata, Pantotheria); *Docodon** (Docodontidae, Trituberculata, Pantotheria); *Didelphis marsupialis, D. paraguanensis, Eodelphis browni,** *Monodelphis domestica* (Didelphidae, Marsupialia, Metatheria); *Borhyaena,** *Prothylacinus,** *Thylacosmilus** (Borhyaenidae, Marsupialia, Metatheria); *Chaetocercus cristicauda, Ch. hillieri, Antechinomys spenceri, Myrmecobius fasciatus, Sminthopsis murina, S. hirtipes, S. crassicauda, S. larapinta, Dasyurus maculatus, D. viverrinus, D. geoffroyii, Dasyuroides byrnei, Phascogale flavipes, P. penicillata, Sarcophilus ursinus, Thylacinus cynocephalus* (Dasyuridae, Marsupialia, Metatheria); *Notoryctes typhlops* (Notoryctidae, Marsupialia, Metatheria); *Isodon obesulus, I. nauticus, Thalacomys nigripes, T. lagotis, T. minor, T. sagitta, T. leucurus, Chaeropus castanotis* (Paramelidae, Marsupialia, Metatheria); *Trichosurus vulpecula, T. caninus, Dromicia concinna, Phascolarctos cinereus, Phalanger maculatus, P. orientalis, Pseudochinus laniginosus notilis,** *Dactyopsila megalura* (Phalangeridae, Marsupialia, Metatheria); *Bettongia lesueri, Hypsiprimnus cuniculus,** *Lagorchestes leporoides, Petrogale xanthopus, P. pearsoni, Onychogale lunata, Thylogale eugenii, Wallabia greyi, W. rufogriseus, Macropus robustus, M. rufus,*

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† Veterinary School, University of Queensland, Brisbane.

M. giganteus, Dorcopsis macleayii (Macropodidae, Marsupialia, Metatheria); *Lasiorhinus latifrons, Phascolomys mitchelli* (Phascolomyidae, Marsupialia, Metatheria); *Deltatheridium** (Deltatheridiidae, Deltatheroidea, Zalambdodonta, Insectivora); *Selenodon** (Selenodontidae, Centetoidea, Zalambdodonta, Insectivora); *Centetes* (Centetidae, Centatoidea, Zalambdodonta, Insectivora); *Zalambdalestes** (Zalambdalestidae, Erinacoidea, Dilambdodonta, Insectivora); *Erinaceus europeus, E. romanicus* (Erinaceidae, Erinacoidea, Dilambdodonta, Insectivora); *Crocidura leucodon, Myosorex johnstoni, Neomys fodiens, Pachyura etrusca, Sorex vulgaris, S. araneus, S. alpinus, S. minutus* (Soricidae, Soricidoidea, Dilambdodonta, Insectivora); *Talpa europea, T. caeca, T. occidentalis, Galemys pyreneicus** (Talpidae, Soricoidea, Dilambdodonta, Insectivora); *Taphozous flaviventris, T. georgianus* (Emballonuridae, Microchiroptera); *Pipistrellus pipistrellus, P. savii, Eptisicus pumilus, E. serotinus, Plecotus auritus, Miniopterus schreibersi, Myotisa adverus, M. mystacinus, M. myotis, Chalinobus moria, Nictophilus geoffroyi, Scoteinus bolstoni, S. greyi, Vespertillo murinus, Nyctalus maximus, N. noctula* (Vespertilionidae, Microchiroptera); *Nyctinomus australis, N. petersi, Chaerophon plicatus* (Mollosidae, Microchiroptera); *Macroderma gigas* (Megadermidae, Microchiroptera); *Rhinolopus ferrum-equinum* (Rhinolopidae, Microchiroptera); *Tillotherium fodiens** (Tillotheridae, Tillodontia); *Notharctus osbornii** (Adapidae, Lemuroidea, Primates); *Anagale** (Anagalidae, Lemuroidea, Primates); *Necrolemur antiquus,** *Nycticebus borneanus, Propithecus diadema, P. coquereli* (Lemuroidae, Lemuroidea, Primates); *Tupaia dasyprocta* (Tupaidae, Lemuroidea, Primates); *Chiromys madagascariensis** (Chiromyidae, Lemuroidea, Primates); *Sinclairella** (Apatemyidae, Tarsioidea, Primates); *Hapale jacchus, Mystax devillei, M. ursulus* (Hapalidae, Plath., Anthropoidea); *Cebus capucinus, C. imitator, C. fatuellus, C. pallidus, Alouatta niger, Macacus rhesus, Macaca mulatta, M. innus, M. nigra, Brachyteles arachnoides, Ateles ater, A. vellerosus, A. marginatus, Saimiri sciurea, Lagothrix humboldti, Papio porcarius, Mesopithecus pentelicus, Semnopithecus entellus ajax, Cercopithecus mona pyrogaster, C. nictictans whitesidei, C. aethiops cynosurus, C. cephus erythrotis, Cercocebus torquatus atys, Colobus polykomos satanas,* *C.p. angolensis, C.p. sharpei, C.p. caudatus, Trachypithecus phayrei, T. pileatus shortridgeii, Presbitis femoralis rhionis, Erythrocebus patus, Mandrillus sphinx* (Cercopithecidae, Catairhinna); *Hylobates noolock, H. larmuelleri, Symphalangus syndactylus* (Hylobatidae, Primates); *Gorilla gorilla, Anthropopithecus tryclodytes, A. cottoni, Simia satyrus* (Simidae, Catairhinna); *Homo sapiens fossilis, H.s. recens* (Hominidae, Primates); *Deltatherium praetriturberculare** (Arctocyonidae, Procreodi, Creodontia); *Mesonyx** (Mesonychidae, Pseudocreodi, Creodonta); *Oxyaena** (Oxyaenidae, Pseudocreodi, Creodontia); *Sinopa rapax** (Hyenodontidae, Pseudocreodi, Creodontia); *Vulpavus protectus** (Miacidae, Fissipedia); *Genetta stuhlmanii; Herpestes ichneumon, Atilax paludinosus robustus, Mungos mungo, Crossarchus obscurus, Ichneumia albicauda, Viverricula malaccensis, Paradoxurus, Myonax canui, Civettictis civetta* (Viverridae, Aeluroidea, Fissipedia); *Hyena crocuta, H. variabilis, H. examina, H. spalea, H. brunea* (Hyaenidae, Aeluroidea, Fissipedia); *Felis catus, F. sylvestris, F. tigris, F. leo, F. spelea, F. concolor, F. pardus, F. leiodon, Lynx lynx, Smilodon neogenus,* S. californieus,* Machairodus cultidriens,* M. paludiens,* Hoplophoneus primaevus,* Nimravus gomphodus,* Dinictis squalidens,* Metailurus minor,* Pardofelis mormorata* (Felidae, Aeluroidea, Fissipedia); *Enhidris marina, Mephitis mephitica, Arctonx callaris, Mustela putorius, M. nivalis, M. erminea, M. robusta, M. lutreola, Martes martes, Meles meles, Lutra lutra, Gulo borealis, Amblonyx cinerea, Tayra barbosa, Ictonyx striata, Poecilogale albinucha, Spilogale lucasana, Conepatus amazonicus* (Mustelidae, Arctoidea, Fissipedia); *Canis aureus, C. lupus, C. lagopus, C. familaris, C.f. grajus, C.f. inostrancevi, C.f. matris optimae, C.f. dingo, C. vulpes, C. adustus, C. mesomelas, Simocyon primigenius,* Cerdocyon thous, Pseudocynodyctis,* Cynodesmus** (Canidae, Arctoidea, Fissipedia); *Euprocyon concrivorus, Potos flavus, Nasua, Zodialestes** (Procyonidae, Arctoidea, Fissipedia); *Ursus arctos, U. maritimus, U. beringianus, U. spelaeus,* Arctodus,* Selenarctos thibetanus, Hemicyon* (Ursidae, Arctoidea, Fissipedia); *Arctocephalus cinereus, A. doriferus, A. forsterii, Otaria byronia* (Otaridae, Pinnipedia, Carnivora); *Phoca vitulina, Hydrurga leptonyx, Leptonychotes weddeli* (Phocidae, Pinnipedia, Carnivora); *Trichechus* (*Odobenus*) *rosmarus* (Odobenidae, Pinnipedia, Carni-

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vora); *Pantolambda bathmodon** (Coryphodontidae, Amblypoda); *Uintatherium laticeps,** *Bathyopsis fissidens,** *Eobasileus** (Uintatheridae, Dinocerata); *Procavia capensis, P. matschei, Dendrohyrax neumanni, D. crawshayi, D. dorsalis* (Hyracidae, Hyracoidea); *Arsinotherium zitteli** (Arsinotheroidae, Embrithopoda); *Maeritherium layonsi* (Maeritheriidae, Maeritheroidea, Proboscidea); *Mastodon pentelici,** *Bunolophodon augustridens,** *B. longirostris,** *B. arensis** (Gomphotheridae and Mastodontidae, Elephantoidea, Proboscidea); *Elephas maximus, E. meridionalis,** *E. primigenius,** *Loxodonta africana* (Elephantidae, Elephantoidea, Proboscidea); *Manatus trichechus** (Manatidae, Halicoriformes, Sirenia); *Orycteropus gaudry** (Orycteropodidae, Tubulidentata); *Phenacodus primaevus** (Phenacodontidae, Condylarthra); *Hyracotherium,** *Eohippus,** *Orohippus, Mesohippus, Protohippus,** *Pliohippus,** *Hipparion minus,** *Equus caballus, E. asinus* (Equidae, Equoidea, Hippomorpha, Perrisodactyla); *Megacerops robustus,** *Telmatherium ultimum,** *Titanotherium ingens,** *Dolichorhinus hyognatus** (Titanotheridae, Titanotheroidea); *Protapirus validus* (Tapiridae, Tapiroidea, Tapiromorpha); *Baluchitherium,** *Ceratorhinus schleiermacheri,** *C. sumatriensis, Coelodonta antiquitatis,** *Diceros pachygnatus, Rhinoceros unicornis* (Rhinocerotidae, Rhinocerotidea, Perissodactyla); *Homacodon vagans** (Homacodontidae, Paleodonta, Aritiodactyla); *Lophiohyus alticeps** (Choeropotamidae, Suina); *Sus scrofa, S.s. ferus, S. erymanthius,** *Babirussa babirussa, Patamochoerus penicillata, Pecari tajacu* (Suidae, Suina, Artiodactyla); *Hippopothamus amphibius* (Hippopothamidae, Suina, Artiodactyla); *Lama huanacos* (Camelidae, Tylopoda); *Dorcatherium crassum** (Tragulidae, Traguloidea, Pecora); *Rangifer tarandus, Alces alces, Hydropatus inermis, Muntiacus vaginalis, M. crinifrons, Capreolus capreolus, Cervus elaphus, C. giganteus,** *Dama dama, Megaceros hibernicus** (Cervidae, Cervoidea, Pecora); *Helladotherium duvernoyi,** *Giraffa camelopardalis, Paleotragus rueni,** *Ocapia johnstoni* (Giraffidae, Cervoidea, Pecora); *Gazella brevicornis, G. thomsoni, Oryx gazella, Tragoceros almathea,** *Paleoryx pallasi et lindermayeri,** *Bison bonasus, Bos primigenius,** *B. taurus, Ovibus moschatus, Capra hircus, C. ibex, Rupicapra rupicapra, Syncerus caffer, Ovis aries, O. argali, Ovicapra ovicapra, Antidorcas marsupialis, Ouerbia montana, O. aequatoria, Raphicerus sharpei, R. campestris, Cephalaphus rubidus, C. spodix, Alcelaphus cocei, Nesotragus livingstonianus, Rhynhotragus damarensis* (Bovidae, Bovidea, Ruminantia); *Sciurus vulgaris, Petaurista nitida, Arctomys dentalis, A. bobac, Mormota mormota* (Sciuridae, Sciuroidea, Rodentia); *Cavia cobaya* (Cavidae, Hystricoidea, Rodentia); *Octodontomys gliroides* (Octodontidae, Hystricoidea, Rodentia); *Proechimys cajennensis, P. xantheolus, Pseudomys hermannsburgensis, Laomys pedunculatus, Leporillus jonesi, Notomys longicaudatus, Uromys soherinii, Myodes (Lemmus) lemmus, Mus musculus, M. decumanus, M. spicilegus polonica, M. (Rattus) rattus, Cricetus frumentarius, Cricetulus atticus, Arvicola amphibius, Microtus agrestis, M. arvalis, M. nivalis, M. ratticeps, M. terrestris, Hydromys chrysogaster, Evotomys glareolus, Apodemus sylviaticus* (Muridae, Muroidea, Rodentia); *Lepus timidus, L. europeus, Oryctolagus cuniculus* (Leporidae, Lagomorpha).

Two main skull types are found, in one of which the stresses set up by the muscle of mastication act on a limited and circumscribed region, while in the other the structure is influenced by more dispersed stresses (Tucker 1954). Between the extremes made by these functional variants is situated the whole range of mammalian skulls.

II. Masticatory Stresses and the Cranial Structure

The bones of the skull are connected so closely that mechanically they form one compound structure. The topography of the stress transmission is indicated by bony formations which involve the whole cranium and make possible the classification of skulls on a functional basis. The lack of a functional classification results from the interest of late nineteenth century comparative anatomy

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in problems of phylogeny and the consequent almost complete absence of functional craniological studies.

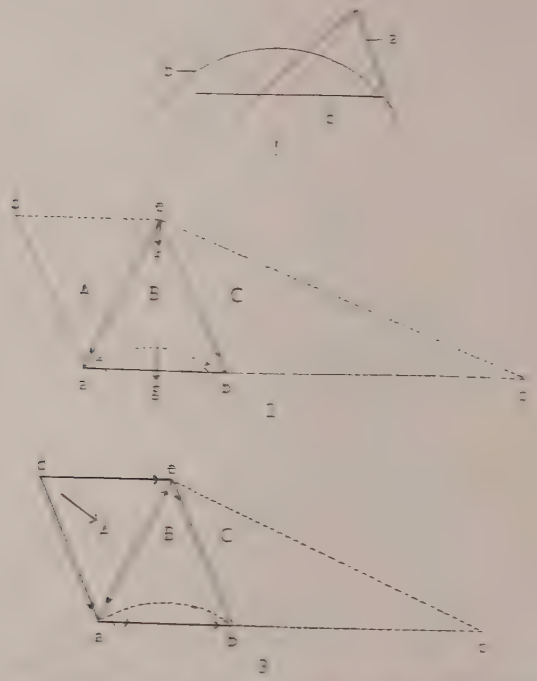


Fig. 1.—Diagrammatic demonstration of various types of the principal cranial arch. *a*, Short cranial arch; *b*, long cranial arch; *c*, flat cranial arch.

Fig. 2.—Diagram of the canine skull. Arrows indicate the vectors engineered from the contraction of the masseter. *A*, posterior triangle, *B*, middle triangle, *C*, anterior triangle, *abe*, principal cranial arch. The middle triangle drawn in line for demonstration of masseteric stresses. The arrows indicate the stresses. The dotted line within the middle triangle shows the position of the zygomatic arch. The large arrows f and g correspond to the forces which arise from the contraction of the masseter.

Fig. 3.—The vectors resulting from the contraction of the temporal muscle. Orientation of diagram and explanation as for Figure 2. The middle and posterior triangle are drawn in line for demonstration of temporal stresses. The arrows indicate the stresses which originate from this muscle. For differences in distribution of stresses between both muscles this diagram should be compared with Figure 2. The arrow in triangle *A* represents the temporal muscle.

The bases of classification proposed here are formed by the distribution of stresses (Tucker 1954) as well as by the development (or lack of development) of structures for transmission of stresses. The most common structure transmitting stresses is an arch which develops between the secondary and tertiary stresses (tertiary in their fixed position) (Tucker 1954).

III. The Principal Arch

The location of the secondary stresses is always the same—the glenoid cavity. The location of the tertiary stresses, according to two main positions of stresses (Tucker 1954), is usually the anterior (premaxillary) part of the alveolar process or the posterior (zygomatic) part of the alveolar process, because the locations of secondary and tertiary stresses are connected by bony structures which transmit the stresses and often have a shape resembling an arch. These transmitting structures are here termed the principal cranial arch (arcus principialis cranii) which may be short or long according to the position of stresses in the particular skull. In skulls in which the transmission of stresses is poor (for instance, shearing stresses), the principal cranial arch is flat. The diagram of the architecture of these arches is presented in Figure 1, in which *a* represents a skull with a short arch (breviarcuate), *b* a skull with a long arch (longoarcuate), and *c* a skull in which the arch is flattened (see also Plate 1, Figs. 1-5).

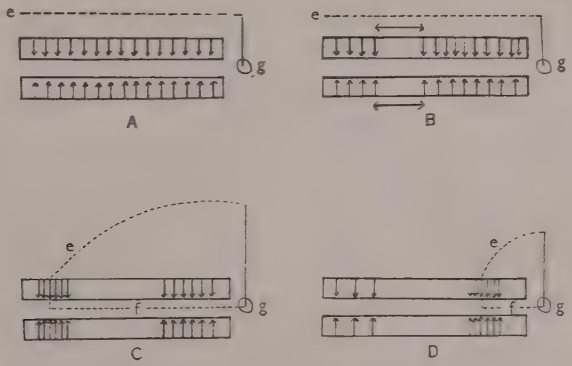


Fig. 4.—The distribution of vectors in different types of skull. *A*, Mesozoic and planoarcuate types. *B*, division of forces and tensions (accumulations). Evolutional stages in some planoarcuate skulls. *C*, longoarcuate type. Grouping of forces in two classes. *D*, breviarcuate type. Main forces accumulated on carnassials. *e*, Morphological character of structure. Principal cranial arch in *C* and *D*; *f*, development of moment in longoarcuate and breviarcuate crania. (The distance between the point of application and the force and the fulcrum.) *g*, Mandibulo-cranial joint.

IV. The Breviarcuate Skull

The arch of the breviarcuate skull ends at a point above the carnassial tooth (Plate 1, Fig. 1; contrast Plate 1, Figs. 2-5). The diagram of distribution of vectors in the principal cranial arch relative to the activities of the masseter and temporal muscle is demonstrated in Figures 2 and 3. The masseter (Fig. 2) acts on the zygomatic arch and through the postorbital ligament on the postorbital process (Fig. 2*e*). The stresses exerted by the masseter are indicated

by arrows. Figure 3 demonstrates the stresses in the principal cranial arch which originate from the contraction of the temporal muscle. These relations will be discussed in detail later.

The existence of the principal arch may be demonstrated morphologically and, as will be shown later, in an experimental way. It could, however, now be indicated that if the decalcified skull is influenced by vectors situated in the glenoid cavity, alveolar process, and in the areas of main insertions of mm. masseter and temporal, the resulting stresses give a pressure between the glenoid cavity and postorbital processes of the frontal bone as well as between the postorbital processes and the carnassial and tensions in the skull floor through the perpendicular lamina of the palatine bone.

V. The Longoarcuate Skull

The longoarcuate type is characterized by the long principal arch which extends from the glenoid cavity to the premaxillary bone. The premaxillary bone in the longoarcuate skull is usually heavily burdened and consequently well developed. The rodents and lagomorphs may be used as examples of the longoarcuate skull (Plate 1, Figs. 2 and 3; compare with Plate 1, Figs. 3 and 5), and Plate 1, Figure 4 (for comparison with Plate 1, Figs. 1 and 2), and Plate 1, Figure 5.

VI. The Planoarcuate Skull

The planoarcuate type is characterized by lack of distinctly shaped cranial arches. The zygomatic arch is horizontal. The teeth are about the same length and roughly similarly shaped. They form two large surfaces which come into contact simultaneously over their whole area (Plate 1, Fig. 5).

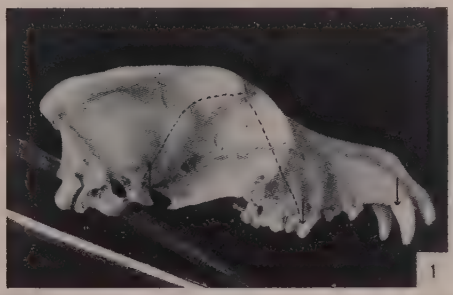
VII. The Primitive Skull

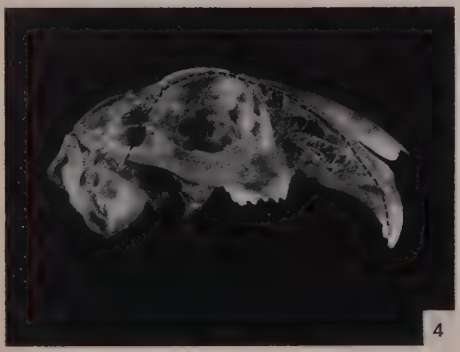
The crania of the Mammalia mesozoica and Insectivora primitiva are skulls with dispersed stresses and of the planoarcuate type; probably this is the primitive type of mammalian skull. Most of the skulls of Marsupialia and Monotremata also demonstrate the features of the planoarcuate type.

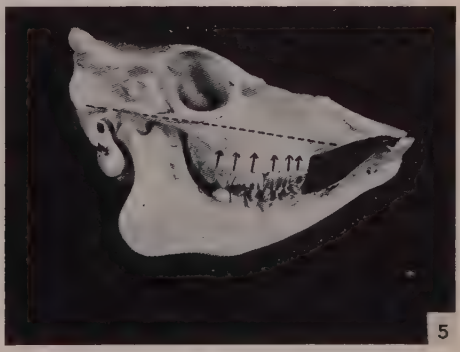
In all these mammals—apart from the varying diets which included Mollusca (*Ornithorhynchus*), insects (*Didelphys, Zalambdalestes*), and plants (*Bettongia*) and apart from the differently shaped dentitions—the food between the maxilla and mandible is crushed by means of the large surface. The teeth may be of equal or almost equal length (*Zalambdalestes, Deltatheridium*); if the teeth are of different lengths (*Bettongia*) one or more of them has a very large occlusal surface. As a consequence, diffuse stresses arise in both alveolar processes.

Skulls with dispersed stresses may differ in the mass of bony tissues, in the morphology and development of dentition, and other local structures, but are always alike in the beam-like character of the maxilla.

FUNCTIONAL AND ANALYTICAL CRANIOLOGY. II







The features of the planoarcuate type are demonstrated by the mandibles of many Mesozoic mammals such as *Eodelphis browni, Dromatherium sylvestre, Amphilestes broderipi,* and *Phascolestes.*

In the planoarcuate skull the transmissive importance of the zygomatic arch is not great. In all primitive and planoarcuate skulls the zygomatic arch according to its static condition is situated horizontally and is often only partially developed.

Probably the most primitive zygomatic arch is, among Mammalia, that of *Zalambdalestes leichei* and of *Deltatheridium* (Abel 1921; Gregory 1951). The zygomatic bone is absent in *Echidna.* In *Ornithorhynchus* it is present only as a small bone in the orbital part of the zygomatic arch. In *Myosorex johnstoni* the zygomatic arch is not developed at all. Among Lipothyphla, only Erinaceoidea have a completely developed zygomatic arch (Weber 1927).

The Centetoidea are characterized by the lack of zygomatic bone and an incompletely developed zygomatic arch (Selenodontidae and Centetidae). Among Soriocididea, a very poorly developed zygomatic arch is demonstrated by Talpidae and an incomplete zygomatic arch by Soricidae (Weber 1927).

VIII. Structural Evolution

Breviarcuate and longoarcuate skulls developed as modifications of the more primitive planoarcuate type (Fig. 4): both evolved in the direction of an accumulation of stresses in limited areas where the accumulation of vectors increased the crushing or shearing effects.

In this way, certain moments of force (f) arise (Fig. 4) which are expressed anatomically by the transmissive structures—arches, ridges, etc. The breviarcuate type, so characteristic of Carnivora, displays the maximum stress in the carnassial (P^4) where the bones of the victim are crushed. Other teeth have accessory tasks only. Even the excellently developed canines (C) rend rather soft tissues, but do not crush hard tissues.

The stresses entering the skull through P^4 have greatly influenced its composition and structure. In breviarcuate skulls, the zygomatic arch is bent dorsally and the maximum effort is assured by its shape and well-developed connection.

The longoarcuate skull evolved in other directions. The dominant stresses are those set up by the incisors in gnawing, and the principal arch therefore extends from the glenoid cavity to the anterior end of the skull. According to this, the whole cranial structure is transformed, for in the zygomatic arch, as in the planoarcuate type, only the stresses developed by the insertions of the masseter remain.

Mechanically, the recent planoarcuate type is only a "new edition" of the mesozoic type. The mandibular and maxillary beams contact simultaneously on the large surface and rest on non-elastic bases (fundaments) so that the local shearing stresses are of the highest order. The massive structure of the splanchnocranium was developed in association with these static and functional conditions while secondary changes are expressed in the orbital regions.

IX. Certain Further Relations of Force

$F \; r \; PP^1$ (position of muscular insertion) (Tucker 1954). However, the development of arches in the longoarcuate, breviarcuate, and planoarcuate skulls influences in a different way the distribution of force. Therefore

$F \; r \; A$ (when A is an arch) and consequently

$A \; r \; PP^1$.

In this way the circle is closed.

X. Acknowledgments

I should like to thank Professor T. K. Ewer, University of Queensland, for his encouragement and interest; and Mr. J. Mack, Director of the Queensland Museum, for providing ready access to specimens.

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Explanation of Plate 1

Fig. 1.—The breviarcuate skull. Laterial view of the dog's skull (right side). The zygomatic arch removed. Principal cranial arch indicated by line.

Fig. 2.—Longoarcuate skull. Lateral view of the cranium of *Uromys scherini* from Queensland Museum (left side). Dotted line demonstrates principal cranial arch (cf. Plate 1, Figs. 1 and 5).

Fig. 3.—Longoarcuate skull. Lateral aspect of cranium of mouse with zygomatic arch removed (left side). The single line demonstrates the principal cranial arch, the double line the principal cranial arch of breviarcuate skull (for comparison with Plate 1, Figs. 1, 2, 5).

Fig. 4.—Longoarcuate skull. Cranium of rabbit (norma lateralis, right side). The cranial arch is indicated by line.

Fig. 5.—Planoarcuate skull. Cranium of cow (norma lateralis, right side). Horizontal zygomatic arch (other arches absent). Arrows and horizontal line indicate dispersion of stresses and morphological character of the whole structure.

STUDIES IN FUNCTIONAL AND ANALYTICAL CRANIOLOGY

III. THE BREVIARCUATE SKULL AND ITS ANALYSIS

By R. Tucker*

[*Manuscript received May* 31, 1954]

Summary

The functional analysis of the breviarcuate skull is based on the cranium of the dog. The tri-triangular structure of the breviarcuate skull is exposed on the level of the palatine bone to the stresses initiated by the masseter. The morphofunctional relations of the palatine bone to the zygomatic arch are discussed and analysed in detail. The most important functional crossroads of the stresses in the breviarcuate skull are the temporal, maxillary, premaxillary, supraorbital, and interparieto-occipital nodes. The vectors which originate directly or indirectly from the masticatory musculature flow through the bony tracts whose pattern bears no relation to that of the individual bones which make up the skull.

The following tracts are described: maxillo-premaxillary (the alveolar process), premaxillo-supraorbital, supraorbito-squamosal, interparieto-occipito-squamosal and occipito-interparieto-supraorbital. The morphofunctional connections of this skull and its functional and structural importance are described and discussed.

I. The Vectors and General Characteristics of the Breviarcuate Skull

The breviarcuate skull is very well demonstrated among carnivora. In the living animal the cranium makes contact with the mandible at three points: the glenoid cavity, the carnassial tooth, and the canines (Plate 1, Fig. 1). It is at these three points that the skull makes contact with the mandible in the living animal. Consequently the main stresses which shape the bones, and indeed the whole skull, arise at these points.

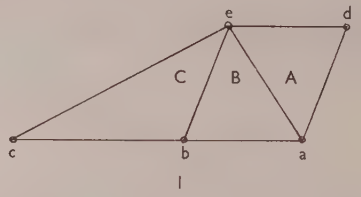
The topography of described regions, direction of stresses as well as their connections with the rigid bony structures, enables us to study the skull as a trabecular structure. The skull observed from the lateral aspect demonstrates the main plane of its structure (Fig. 1; Plate 1, Fig. 1).

The posterior triangle *A* is formed by the connections between the nuchal crest, sagittal crest, postorbital process, and glenoid cavity and the bony tracts connecting them.

The middle triangle *B* is formed by the anterior margin of the orbit and its elongation towards the carnassial (dens lacerans) by the perpendicular lamina of the palatine bone and by the antero-inferior side of the posterior triangle.

* Veterinary School, University of Queensland, Brisbane.

The anterior triangle *C* is created by the alveolar process of the maxillary bone, by the premaxilla, and by the superior edge of the maxilla running back to the post-orbital process.



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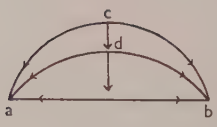

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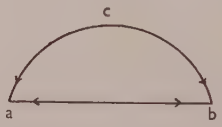

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Fig. 1.—Diagram of the breviarcuate skull. *A*, posterior; *B*, middle; *C*, anterior triangle. *a-d*, Crista nuchalis; *d-e*, crista sagittalis and crista frontalis; *e-c*, superior margin of maxilla; *c*, canine and incisors; *c-b*, alveolar processes of maxilla; *e-b*, anterior margin of the orbit; *e*, postorbital process; *a*, glenoid cavity; *b*, carnassial; *a-b*, perpendicular lamina of the palatine bone.

Fig. 2.—Diagram of the vectors in the principal arch of the skull initiated by the action of the masseter. The horizontal line represents the perpendicular lamina. *a c b*, The principal cranial arch; *a d b*, the zygomatic arch. The action of masseter indicated by the arrows at *c* and *d*. Small arrow—the distribution of secondary stresses.

Fig. 3.—Diagram of stresses in the perpendicular lamina of the palatine bone. The horizontal line demonstrates the perpendicular lamina while the arch represents principal cranial arch. Arrow at the arch shows the stresses which induce vectors (marked by arrows in the base of the arch).

The balance of the whole structure may differ according to functional changes. The stress is most often applied at *a* and *b* (Fig. 1; Plate 1, Fig. 1). Vectors of secondary importance arise along the alveolar processes of the maxillary bone. In every movement of the mandible a vector arises in the glenoid cavity. Because of the importance of vectors at *a* and *b*, the regions of the glenoid cavity and carnassial may be considered as the main points of the skull —the anterior point being at the base of the anterior end of the zygomatic arch (carnassial) and the posterior point at the posterior end of the zygomatic arch (glenoid cavity). At *c*, *d*, and *e* are situated the points of secondary importance.

At the points *a*, *b*, and *c* (Fig. 1) the stresses are created by the bone and teeth of the mandible, while in *d* and *e* they are created in the posterior part of the sagittal crest and in the postorbital process, by the action of the masticatory muscles. The temporal muscle exerts its forces especially at the node *d*. In this paper the term "node" refers to a thickened bony region of limited extent, whose existence seems to be determined by a local concentration of stresses. The stresses set up by the masseter in the zygomatic arch are transmitted to the postorbital process by the postorbital ligament.

The whole system may be influenced by:

(1) The forces exerted by m. masseter. These act through the zygomatic arch at *a* and *b* and through the ligamentum postorbitale at *e*.

(2) The stress initiated from temporal muscles inserted in *d*.

(3) The simultaneous activity of both muscle systems; this is the most common condition.

All parts of the system are bilateral except the sagittal crest, which is the median structure common to both.

It is practically impossible to make an exact estimation of the stresses in different parts of the skull owing to the complicated curvatures of the different bars and their position in various planes. But it would be possible to find the tensions and compressions together with their origins, and hence gather additional knowledge about the relationships of shape, function, and structure. However, up to date this has not been attempted.

II. Morphofunctional Relations of the Palatine Bone

This primary analysis of the influence of the masticatory muscle may now be expanded. The contraction of the masseter initiates stresses simultaneously at three points. The greater part of its insertion is situated on the zygomatic arch, hence vectorial tensions are radiated through the arch structure to *a* and *b* (Fig. 1). If the zygomatic arch is lowered even slightly, the postorbital process *e* is automatically overloaded because the postorbital ligament is situated between the strong postorbital process and zygomatic arch. It is evident from the above that the triangle *a, b, e* is completely under the influence of the masseter.

In other words, the masseter muscle tends to fracture the cranium through the middle triangle (Fig. 2). The stresses in arches *acb* and *adb*, representing the principal cranial arch and the zygomatic arch, accumulate in the main points *a* and *b* (Figs. 2 and 3) and are resisted at *a* by the perpendicular lamina of the palatine bone. Accordingly, the perpendicular lamina is stretched (Fig. 3). The involvement of the perpendicular lamina of the palatine bone into the functional system of masticatory muscles may explain its development in the breviarcuate skull. No strong muscles occur near the perpendicular lamina. The strongest are pterygoid muscles which are connected with the pterygoid bones and partially with the sphenoid bone. The elongated shape of the perpendicular lamina which created the naso-pharyngeal duct (or space) and also its tendency to penetrate into the region of the sphenoid result from the tensorial stresses (between *a* and *b*, Fig. 2) discussed above.

In close relationship to these tensorial stresses induced in the palatine bone is its penetration into the orbit as far as the median orbital crest (Plate 1, Fig. 2, arrow). This structure has a functional value and, in the author's opinion, its position justifies the name of median orbital crest. In certain animals of prey it is very strong. In the same way the palatine connects with the frontal and lacrimal bones as well as with the zygomatic process of the maxillary bone.

The latter takes the main portion of the stresses exerted on the molar bone which is directly pressed and caught such are the maxillary bone. Plate 1 Fig. 2. The bundles of the palatine bone surround this part of the maxilla in the form of a triangle. This forms the maximally extensive connecting surface between the two bones as well as allowing for the most favourable conditions for articulation.

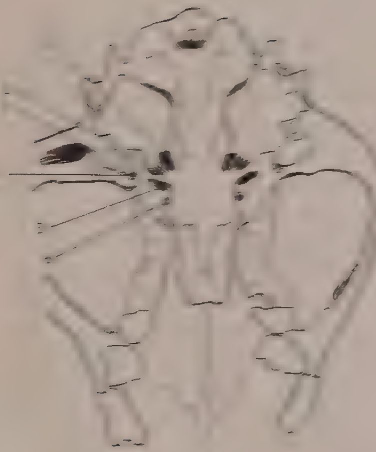
B. Structure of the stress-bone

In various parts of the palato-palatine region the morphology of the osseous bundles differ. The sphenoid bone is the first place where the articular face of the zygomatic process of the squamosal in the perpendicular lamina of the palatine bone, and in the ventral plate. It is situated between the posterior endings of the main arches of the skull. The morphology of the pterygoid shows the existence of various stresses in the skull that between the anterior and posterior ends of the right and left principal arches of the skull. A histological and morphological peculiarity is created by the competition between these stresses and those which are set up by the growth of the bone tissue enlargement the soft or connective tissue bone. In the endosteal period the latter stresses have a different polarization and accordingly they modulate the area between the osteo-genetically directed stresses in the skull and cartilage.

The continued influence of these factors causes the growth tendencies of the main stresses in the region of the sphenoid bone. The maxillae have the answer to the maxillae that the zygomatic process of the squamosal bone corresponds the stress in the body of the sphenoid bone is transmitted to a ventral and especially to the ventral wings (ventro-sphenoid). The number of stresses from the mandibular joint towards the sphenoid and palatine is altered by two different shaped trabeculae and their ends. These are the posterior Plate 1 Fig. 1a and anterior Plate 1 Fig. 1b trabeculae. The former is situated between the internal condylar (or lateral pterygoid) and internal ends Fig. 4 Plate 1 Fig. 1 and 4. The trabecula runs the perpendicular process of the squamosal bone and the perpendicular lamina of the palatine bone. The internal margin of the trabecula commences on the perpendicular lamina of the palatine Fig. 4 Plate 1 Fig. 1. On the commencement a notice also remaining the pterygoid area Plate 1 Fig. 4. Another more massive trabecula trabecula anterior situated rougher anterior-lateral to the first Fig. 4 Plate 1 Fig. 1. a lateral pterygoid or the internal side posterior and to the foramen ovale. The anterior margin of the trabecula continues the anterior margin of the zygomatic process of the squamosal bone and anteriorly continues into the temporal wing of the sphenoid bone (alisphenoid). The trabeculae as well also are connected at the basal part of the zygomatic process by a trabecula formed from the posterior basilaris connections. Fig. 4 Plate 1 Fig. 1d. The foramen is situated between the perpendicular lamina of palatine on the one side and the foramen ovale and the sphenoidal bone on the other.

The medial trabecular connections run between the foramen ovale and posterior and foramen Fig. 4 Plate 1 Fig. 1c.

The brachium conjunctivum anterior forms a relatively broad lamina between the anterior trabecula and the posterior end of the palatine bone (Fig. 4; Plate 1, Fig. 3), thus forming the alar commissure.



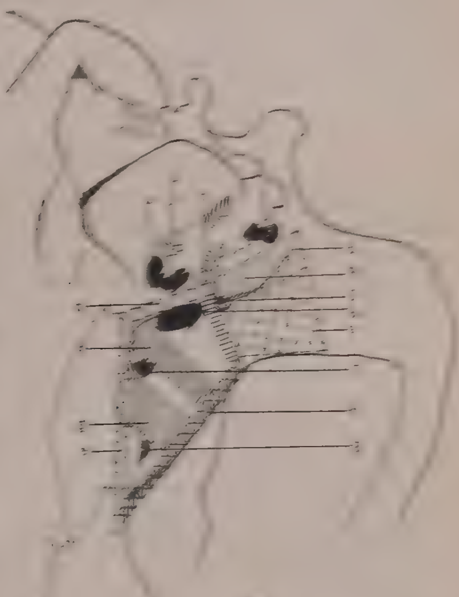


Fig. 4.—Topography of the cranial foramina which limit the trabeculae and brachia conjunctiva. 1, Foramen caroticum; 2, eustachian opening; 3, postglenoid process; 4, petrotympanic fissure of Glaser; 5, foramen ovale; 6, foramen alare posterior.

Fig. 5.—The structural scheme of the sphenoid region of the dog. The basic elements are shown on the illustration of the sphenoid region of the dog. Diagram does not show the proportions but the relations of single elements towards each other and the structural composition of the sphenoid region. 1, meatus acusticus externus; 2, bulla ossea; 3, tuba-auditiva eustachii; 4, foramen caroticum externum; 5, fissura petrotympanica Glaseri; 6, foramen ovale; 7, foramen alare posterius; 8, foramen alare anterior; 9, processus postglenoideus. a, articular facet of glenoid fossa; b, area of postglenoid process; c, posterior brachium conjunctivum; d, trabecula posterior; e, perpendicular lamina of the palatine bone; f, medial brachium conjunctivum; g, anterior brachium conjunctivum; h, trabecula anterior.

IV. The Structural Scheme of the Sphenoid Region

The morphological picture described above can be reduced to its most simple mechanical elements, namely, two trabeculae and the three conjunctive bars between them (Fig. 5). Foramina, important as they may be from neurological, embryological, or comparative points of view, mechanically are only the empty spaces whose single value is in breaking or limiting the routes of stresses.

In the light of the functional analysis presented above, the sphenoid region develops under the influence of stresses which originate from the vectors in the principal cranial arch (cf. Figs. 2 and 3). Hence

$$S^1 \; r \; A$$

when S^1 is the sphenoid region. On the other hand,

$$A \; r \; PP^1$$

(Tucker 1954*b*). Therefore

$$S^1 \; r \; PP^1.$$

V. The Zygo-sphenoidal Curvature

The above discussion has involved firstly the zygomatic region, and secondly the sphenoid region. It is possible that the zygomatic arch works partially in the same way as bar *a-b* (Fig. 2). As a consequence of its position and structure, each zygomatic arch opposes the wider extension of the main points of the skull in a horizontal plane on the opposite side. The importance of the anterior conjunctival crest which is formed by the anterior margin of the anterior trabecula must be considered with the tensions in the zygomatic arch as well as in the bar *a-b*. The zygomatic process observed from above and in lateral view shows the connections between the ventral margin of the zygomatic process, the anterior conjunctival crest, and the orbito-alar lamina (septum) (Fig. 5). All these structures are parts of the same zygo-sphenoidal curvature. The curvature has its highest point above the mandibular joint, its peripheral rami running towards the palatine bone and the malar bone. The stresses pass along this zygo-sphenoidal curvature, the larger stress into the palatine bone and the smaller into the malar bone (Plate 1, Fig. 2*c*).

The structures here termed orbito-alar lamina (septum) and zygo-sphenoidal curvature are named after their topographical localizations.

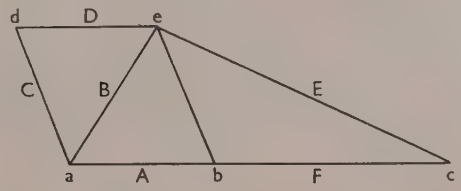
VI. Mechanical Significance of the Anterior Conjunctival Crest

The anterior margin of the anterior trabecula which forms a less distinct structure than the margin of the posterior trabecula (posterior conjunctival crest, Fig. 4; Plate 1, Fig. 3) nevertheless has a functional significance. Along this structure occurs the separation of stresses into two distinct planes. At the anterior conjunctival crest meets the plane of the anterior trabecula which, together with the sphenoid, limits the brain case inferiorly, and the plane of the squamosal bone, which makes part of the lateral wall of the brain case. An important functional link is created by the sphenoid; this bone connects with the posterior trabecula and with the anterior conjunctival brachium on the one side, and with the perpendicular lamina of the palatine bone on the other, and transmits the tensions into the orbit.

VII. The Nodes and Tracts

It appears that the most convenient nomenclature for describing biotic structures is the topographical one. Accordingly, the node *a* will be described

as the squamosal node, node *b* as the maxillary node, node *c* as the premaxillary node, node *e* as the supraorbital node, and node *d* as the interparieto-occipital node (Fig. 6; Plate 2, Fig. 1). The nodes are connected by tracts (Fig. 6; Plate 2, Fig. 1) which will be called after the names of nodes between which they



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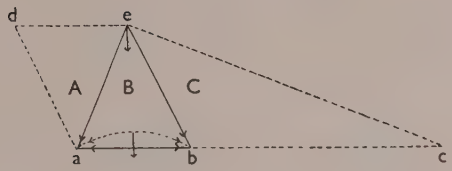

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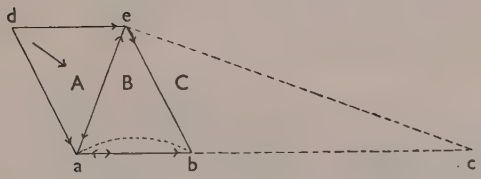

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Fig. 6.—Topography of nodes and tracts (cf. Fig. 1). *a*, Squamosal node; *b*, maxillary node; *c*, premaxillary node; *d*, interparieto-occipital node; *e*, supraorbital node; *A*, squamoso-maxillary tract; *B*, squamoso-supraorbital tract; *C*, squamoso-occipito-supraorbital tract; *D*, interparieto-occipito-supraorbital tract; *E*, supraorbito-premaxillary tract; *F*, premaxillo-maxillary tract.

Fig. 7.—Vectorial analysis of stresses originated from the contraction of the masseter. *A*, posterior triangle; *B*, middle triangle; *C*, anterior triangle; *a-e*, as Figure 6. The dotted arch represents the zygomatic arch.

Fig. 8.—Vectorial analysis of the stresses originated from the contractions of the temporal muscle. *a*, Squamosal node; *b*, maxillary node; *c*, premaxillary node; *d*, interparieto-occipital node; *e*, supraorbital node. In contrast to the masseter, which acts upon the middle triangle only (Plate 2, Fig. 1), the temporal muscle sets up stresses in the posterior and middle triangles. The arrow indicates the main direction of the fibres of the temporal muscle. The dotted arch represents the zygomatic arch.

are situated. Therefore, the squamoso-maxillary tract (Plate 1, Figs. 3 and 4; Plate 2, Fig. 1) lies between the squamosal and maxillary nodes on the floor of the skull, the squamoso-supraorbital tract between the squamosal and supraorbital nodes, and so on.

VIII. The Temporo-masseteric Relations

The structural adaptations are more advanced in the supraorbito-maxillary tract (*b-e*) (Fig. 5) than in the supraorbito-squamosal tract (*e-a*) (Fig. 5). The interpretation of this phenomenon will be made after the analysis of the morphofunctional connections of the temporal muscle has been completed.

Another factor influencing the supraorbito-maxillary tract is the contraction of the masseter during which the tensions in bar *a-d* and *d-e* are near zero (Tucker 1954*b*, Figs. 2 or 3). The distribution of vectors by the contraction of the temporal muscles is quite different from that originating from the action of the masseter. These differences are demonstrated in Figures 7 and 8.

The contraction of the temporal muscle creates tensile stresses in the squamoso-supraorbital tract (Fig. 8*a-e*). The masseter, on the contrary, sets up pressure stresses in this tract. The opposed actions of the two muscles have important morphological consequences and will be discussed in detail later. Other tracts exposed to the stresses created by the contraction of the temporal muscles are compressed (squamoso-occipito-interparietal tract (Fig. 8*a,d* and Fig. 6*c*) and supra-orbito-maxillary tract (Fig. 8*e-b* and Fig. 6)). The greatest stresses are located in the squamo-interparieto-occipital tract.

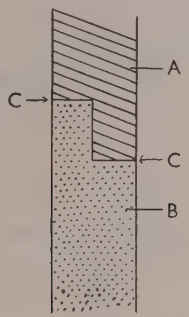
According to this analysis the fates of the squamoso-interparieto-occipital and interparieto-supraorbital tracts are connected closely with the development, topography, and function of the temporal muscles. The relations between the sagittal crest, which forms part of the occipito-interparieto-supraorbital tract, and the temporal muscle, are known as the characteristic development of the sagittal crest in carnivorous mammals.

IX. The Role of the Temporal Muscle

Contraction of the temporal muscle tends (*a*) to pull the parietal away from the skull wall, so that stresses are set up especially in its dorsal sutures, (*b*) to set up stresses in the occipito-interparieto-supraorbital and occipito-interparieto-squamosal tracts. In addition the temporal fascia inserts upon the zygomatic arch as well as on the sagittal crest; thus these two structures are more closely connected functionally than their distance and their separation by the temporal fossa would lead one to suppose. It is possible that contractions of the masseter may bend the zygomatic arch and so through the fascial connection set up stresses in the sagittal crest and elsewhere, reinforcing the action of the temporal muscle. The integration of function between the two muscles is further emphasized by the insertion of parts of the temporal on the masseter.

In the hindmost part of the skull (Plate 2, Fig. 2) three well-developed bony walls form three arms which join in the occipito-interparietal node. The interparieto-occipital node is a single median node which binds the whole cranial structure. Accordingly none of the other sutures in the sagittal plane of the skull is developed as strongly as the connections in the interparieto-occipital node. In fact, the internasal suture forms only a loose connection and the interfrontal suture only in its posterior part close to the sagittal crest has dentate sutural formations. Only the parietals are joined by a strongly

formed, ridged suture. Further, the development of the synostosis between the interparietal bone and the squama of the occipital bone indicates the tendency to very strong binding of the symmetrical elements in the interparieto-occipital node. By the synostosis, the interparietal bone is changed functionally into a process of the occipital bone, which thus continues into the roof of the skull. On the other hand, the development of these formations is influenced by the connections of the interparietal bone with the cervical muscles. The cervical muscles have insertions on and in the neighbourhood of the nuchal crest. The spinal myology and its cranial relations are, however, out of the scope of this paper.



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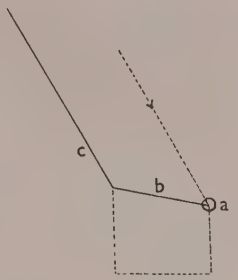

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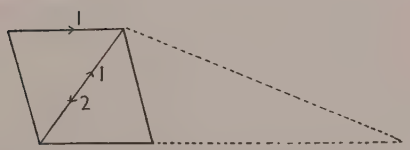

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Fig. 9.—The construction of the rebated joint. *A-B*, bony components; *C*, connecting surface.

Fig. 10.—Diagram illustrating the development of the torque in the squamosal node (for comparison with Plate 2, Fig. 1*a*, *b*, and Plate 2, Fig. 4). *a*, glenoid cavity; *b*, sustentaculum. *c*, occipito-interparieto-squamosal tract. These points on the diagram correspond to the same markings in Plate 2, Figure 4, and illustrate the distance between the lower endings of the squamoso-supraorbital and squamoso-interparieto-parietal tracts.

Fig. 11.—Diagram of stresses in the squamoso-supraorbital tract caused by the simultaneous action of both masticatory muscles. 1, The vectors caused by the temporal muscle; 2, the vector caused by the masseter muscle.

The structure of the interparieto-occipital node is developed mainly under the influence of the temporal muscle. No other muscle in the head is able to set up laterally directed stresses to such an extent as the temporal muscle. Apart from the temporal muscle, only the masseter need be considered. But the insertions of the masseter are situated relatively low, mainly on the zygomatic arch, which transmits the stresses directly into the base of the cranium. Owing

to these conditions, even the strong contraction of the masseter cannot give rise to very large tensions in the roof of the skull.

The working conditions of the temporal muscle are different. Its insertions are situated high up. Some are even on the sagittal crest. The contraction of the temporal muscles directly initiates tensions in the roofing parts, and determines the stronger union of bones in the posterior part of the skull roof.

The strong development of the interparieto-occipital node of the upper border of the occipital bone and of the tracts terminating in the interparieto-occipital node is also influenced by the action of the temporal muscle.

X. The Interparieto-occipito-squamosal Tract

Most of the structural and functional units in the skull are formed from bones of various origin. This is true also for the interparieto-occipito-squamosal tract, which has the upper part built from the occipital bone and the rest from the squamosal (temporal) bone. The axis of this tract is roughly parallel to the axis of the temporal muscle. Therefore the main stresses induced by the temporal muscle are transmitted along it. The junction between the occipital and temporal bones develops as a rebated joint (sutura gradica) (Fig. 9; Plate 2, Fig. 3*c*). Consequently the stresses are transmitted into successive functional elements in a way similar to that of the fronto-maxillary suture in the frontal process of the maxillary bone.

The relations of the squamosal node to the occipito-interparieto-squamosal and squamoso-maxillary tracts appear to have certain morphological and functional similarities, based chiefly on the indirect transmission of the stresses. Between the squamosal end of the interparieto-occipito-squamosal tract, which terminates in a squamosal tubercle (Fig. 10; Plate 2, Fig. 3*a*; Plate 2, Fig. 4), and the zygomatic process of the squamosal bone, is present a specific ridge—a sustentaculum (Fig. 10; Plate 2, Fig. 3*a,b*; Plate 2, Fig. 4). The morphological peculiarity is apparently due to the existence of stresses other than those mentioned above. The postglenoidal region is a "crossroads" of different stresses from the early developmental stages. In this part of the head is formed the otic vesicle and eventually the internal and middle ear. The perinodal region of the squamosal bone is shaped under the influence of the acoustic organ and accordingly these stresses necessarily take part in the formation of the sustentaculum. Perhaps it is a good illustration of the fact that every bone is a compromise (Murray 1936).

XI. The Interparieto-occipito-supraorbital Tract

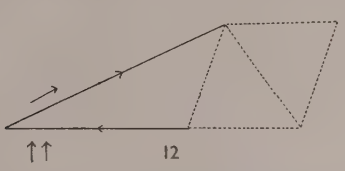
The mechanics of this tract are influenced to a high degree by the temporal muscle. One of its most important parts is the sagittal crest. The occipital part of the crest is rather thin, and it becomes thicker only at the level of the junction between the crest and the interparieto-parietal suture. Posteriorly, the interparieto-parietal suture runs below the sagittal crest, climbing onto the crest in its anterior third. Through this connection are transmitted the stresses from the interparietal bone to the parietal bone.

By virtue of these topographical relations, the parietal bone becomes the route of stresses which pass through the sagittal crest towards the frontal crest (considering only the stresses in the sagittal plane).

To sum up, the sagittal crest has three parts (Plate 2, Figs. 3 and 5):

(1) A part made by the interparietal bone;
(2) A part in which both parietals and the interparietal take part;
(3) Most anteriorly situated, the parietal part which shows a tendency to division.

This last tendency is indicated by the development of the median groove of the sagittal crest. Now the stresses flow in both halves of the sagittal crest (Plate 2, Fig. 3). The frontal crests join the brachia of the sagittal crest by means of the sutura gradica. It should be pointed out that the rebated joint (sutura gradica) is developed wherever the influence of the temporal muscle is felt.



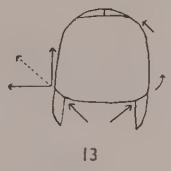


Fig. 12.—Diagram of the anterior triangle, showing main stresses in this region. The large arrows show the rotatory vectors.

Fig. 13.—Diagram of vectors in the transverse plane of the anterior triangle. Explanation in text.

XII. The Torus and the Squamoso-supraorbital Tract

The frontal crests are much broader in their anterior than in their posterior part. The presence of the postorbital fossa (Plate 2, Fig. 3*e*), the anterior part of the temporal fossa, accentuates the convex structure of the crested part of the frontal bone, which is functionally connected with the supraorbito-squamosal tract. The route of the main stresses is in the neighbourhood of the frontal crest and the special structure—the "torus" (Plate 2, Fig. 3*f*). The torus is limited anteriorly by the frontal crest and forms the median wall of the postorbital fossa. The morphology of the parietal bone and its connections with the supraorbital node as well as with the supraorbito-squamosal and supraorbito-maxillary tracts create a rather difficult complex. The value and development of this structure is demonstrated by the analysis of opposing stresses shown on the diagram (Fig. 11).

When the temporal muscle contracts, the stresses which it sets up in the supraorbito-squamosal tract are tensile, but when the masseter contracts, they are compressed. Thus, when both muscles contract, the two sets of strains tend to cancel each other. For this reason, the tract is morphologically undeveloped.

XIII. The Anterior Triangle

The plan of the carnivorous skull is completed by the anterior triangle (Figs. 1, 11, 12; Plate 1, Fig. 1), which is formed by the maxillary and premaxillary bones. The base of the anterior triangle is shaped by the maxillo-supraorbital tract. Other tracts of the triangle are:

(1) the maxillo-premaxillary tract (Fig. 1*bc*) continuing forward from the squamoso-maxillary tract, and

(2) the premaxillo-supraorbital tract, which runs parallel to the superior edge of the maxilla (Figs. 1*ce*, 12).

The sources of stresses in the anterior tract are similar to those in the median and posterior triangles, viz. the masseter and temporal muscles, which act on the mandible.

The simultaneous action of masticatory muscles when the food is present between the maxillary and mandibular rows of teeth sets up stresses in and perpendicular to the premaxillo-maxillary tract. As a result of these stresses, the anterior triangle has a tendency to rotate upward (Fig. 12). Consequently the stresses in the premaxillo-maxillary tract are partly similar to those of the squamoso-maxillary tract because the tendency to upward rotation sets up tension stresses in both tracts. In general, the stresses present in the anterior triangle provoke the increase of stresses in the squamoso-maxillary and the interparieto-occipito-supraorbital tract by addition of stresses in the anterior triangle to the stresses in the median and posterior triangles.

Other stresses which are characteristic of the development of structures in the facial region are the laterally directed stresses arising between the upper and lower rows of teeth. The mandibular teeth have a more medial position than the maxillary teeth. Accordingly, the pressure from the mandibular teeth is directed dorso-laterally (Fig. 13). The horizontal and vertical components of the above-mentioned stresses and forces differ according to their level in the anterior triangle. The vertical component is led dorsally in the vertical plate of the maxilla but as the maxilla turns horizontally to meet the nasal this component is led into a horizontal orientation, and the components on the two sides tend to press the nasal bones together (Fig. 13).

XIV. Acknowledgment

My thanks are due to Professor J. F. A. Sprent, University of Queensland, for discussing the problems connected with the paper.

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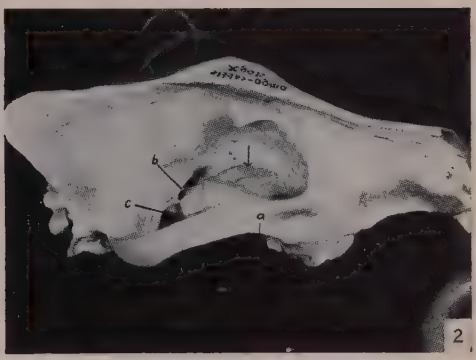
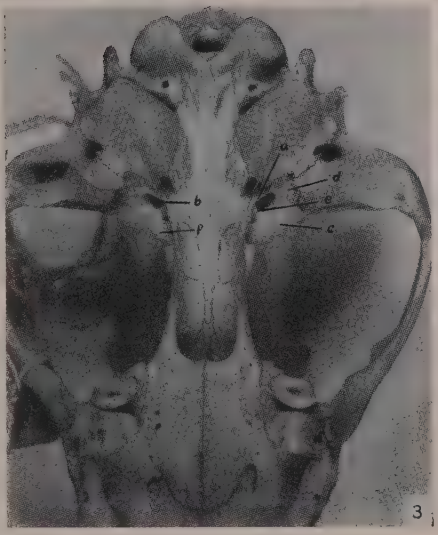
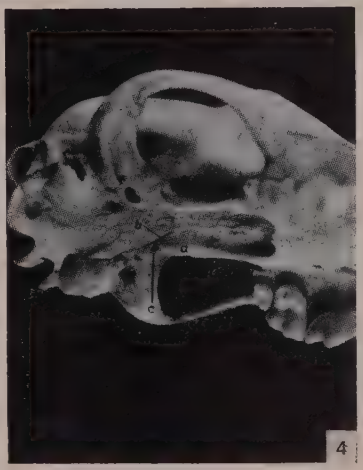
FUNCTIONAL AND ANALYTICAL CRANIOLOGY. III







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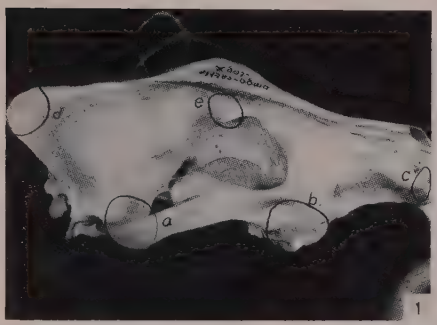

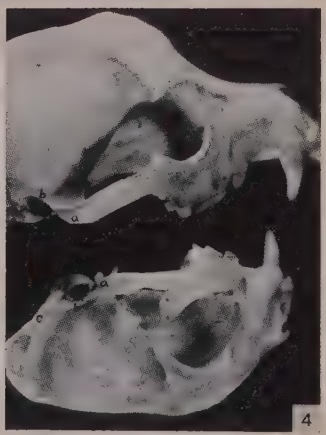

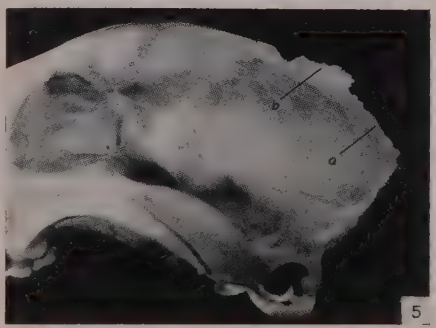

Explanation of Plates 1 and 2

Plate 1

Fig. 1.—The skull of the dog (lateral view, left side). The zygomatic arch removed. The line shows the anterior, middle, and posterior triangles. Other explanations as for Figure 1 in text.

Fig. 2.—The skull of the dog (lateral view, right side). Arrow indicates the medial orbital crest. *a*, Inferior ramus of the zygomatic bone; *b*, lamina (septum) orbito-alare; *c*, zygo-sphenoidal curvature.

Fig. 3.—The skull of the dog. Ventral view. Connections around the squamosal node. *a*, Posterior trabecula; *b*, crista conjunctivale posterior; *c*, anterior trabecula; *d*, posterior brachium conjunctivum; *e*, medial brachium conjunctivum; *f*, anterior brachium conjunctivum.

Fig. 4.—Skull of the dog. Pterygoid bone removed to show the connections between the trabecula and the perpendicular lamina of the palatine bone. *a*, The perpendicular lamina; *b*, the petrosal sulcus; *c*, the connections shaped as a crest (crista conjunctivale posterior).

Plate 2

Fig. 1.—Topography of the nodes in the dog (lateral view of the skull, right side). *a*, Squamosal node; *b*, maxillary node; *c*, premaxillary node; *d*, interparieto-occipital node; *e*, supraorbital node.

Fig. 2.—The occipito-interparietal node.

Fig. 3.—The structure of the sagittal crest and the occipito-interparieto-temporal tract in the dog. *a*, Squamosal tubercle; *b*, sustentaculum; *c*, rebated joint (sutura gradica); *d*, frontal crest; *e*, posteristal fossa; *f*, torus; *g*, postorbital process. In the orbita is visible the median orbital crest.

Fig. 4.—The sustentaculum and glenoid cavity in the dog (above) and cat (below). The orientation of the upper skull corresponds with the orientation of the diagram (Fig. 10). Explanation as Figure 10.

Fig. 5.—Parietal component of the sagittal crest (dog's skull, lateral view, left side, the interparietal bone removed to demonstrate the outline of the parietal bone within the sagittal crest). *a*, Connection with the interparietal bone; *b*, anterior portion of the parietal bone composing part of the sagittal crest.

STUDIES IN FUNCTIONAL AND ANALYTICAL CRANIOLOGY

IV. MORE EXTREME FORMS OF THE BREVIARCUATE SKULL

By R. Tucker*

[*Manuscript received May* 31, 1954]

Summary

The breviarcuate character of the dog's skull is not very pronounced. More advanced adaptations among the carnivorous mammals towards the full development of the breviarcuate skull are described and analysed in detail.

When we compare the transformations in the zygomatic arch region of more strongly breviarcuate skulls with that in the skull of Canidae, it is clear that they are bound up with the muscular differences and involve: the lesser distance between the canines and the zygomatic arch; the greater strength of fronto-zygomatic connections; the larger surfaces connecting the malar bone and maxilla; the fact that the zygomatic arch is not divided at its connection with maxilla; the development of the lamellar system and the presence of the gomphus (a non-mobile joint in which one bone fits into a groove or cavity in the other) in the maxillo-malar junction. The detailed structure of these connections is described. Also described is a shallow depression on the malar bone in which lies the end of the zygomatic process of the squamosal bone, with its function and connections. Most of the analyses are based on the skull of the domestic cat.

I. The Character of Specializations in the Breviarcuate Skull

The cranium of Canidae has craniological features which are similar to the planoarcuate and longoarcuate types of skull. This is in agreement with other features of Canidae such as the quite 'ungulate' ability to run. The skull of the cat demonstrates the more advanced changes in the skull morphology connected with the action of masticatory musculature. Especially characteristic are the nodes—premaxillary, maxillary, supraorbital, squamosal, and interparieto-occipital. The main transformations are shown in the shortening of the facial region and the main arch of the skull as well as in the changes in the squamosal, occipital, and interparietal bones. The type of skull with circumscribed or focused stresses is exemplified in the skull of the cat. The stresses are more concentrated than in the Canidae; the molars are situated close to the zygomatic arch; the canine and its surroundings develop to a greater extent, and the distance between molars and canines is less. The canine is located almost in the region of the zygomatic arch (Plate 1, Fig. 1). The connections between the supraorbital node and the zygomatic arch are stronger than in Canidae. The postorbital process of the frontal bone approximates the frontal process of the malar bone. In consequence, the postorbital ligament is much shorter (Plate 1, Fig. 2).

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The connection between the maxilla and malar bones is constructed in a different way from that in the dog. The anterior end of the malar bone is not forked as it is in the dog (cat, Plate 1, Figs. 3, 4; dog, Plate 1, Figs. 5, 6). However, on its external face there is present a distinct zygomatic line (Plate 1, Figs. 1*a*, 4) which, after passing the postorbital process of the malar bone, suddenly turns inferiorly and forms a knee of the zygomatic line (Plate 1, Fig. 1). The most posterior part of the zygomatic line runs roughly parallel to the superior margin of the malar bone.

The chief specializing changes in the region of the zygomatic arch are:

(1) The change in the connection between the postorbital process of the frontal bone and the frontal process of the malar bone (Plate 1, Fig. 2);

(2) The enlargement of the conjunctive sutural and lamellar surface between the malar bone and the maxilla (cat, Plate 1, Figs. 3 and 4, for comparison, dog, Plate 1, Figs. 5 and 6).

This latter sutural and lamellar connection in the cat occupies the whole junction from the lacrimal to the zygomatic process, whilst in the dog it is limited to the inferior brachium of the malar bone only. In the dog the sutural lamellae are poorly developed and the gomphosis (a peg and socket joint) is partially replaced by a rebated joint (sutura gradica).

A gomphosis is formed in all cases in which (*a*) relatively large forces are transmitted, (*b*) the two components of the suture must be held firmly without movement by the suture itself, virtually without help from other sutures, bones, or soft structures. If the first condition exists, without the second as a result of the support given by neighbouring bones, a sutura gradica is formed.

II. The Maxillo-malar Junction

The characteristic formations here are:

(1) The presence, in the posterior region of the suture and sutural lamellae resembling those in the inferior brachium of the malar in Canidae. These parts are homologous.

(2) The presence of the gomphosis which is developed between the maxilla and the malar bone.

Corresponding to the gomphus (the "male" component of the gomphosis) is the groove in the maxillary bone (Plate 2, Figs. 1, 2, 3). The borders of the malar bone and the lacrimal bones form a rebated joint (sutura gradica) (Tucker 1954*c*). Under the stresses arising from the contraction of the masseter, the zygomatic arch tends to be flattened, with its anterior end moved forward (and its posterior end backward). The maxillary gomphus, as well as the inward and upward turn of the suture at its anterior end, opposes this tendency (Plate 2, Figs. 1, 2, 3).

The malar bone runs into the maxilla and the sutural surfaces of the malar and maxillary bones correspond. The posterior part of the sutural surface of

the malar bone is situated between the posterior margin of the body of the malar bone and the zygomatic line (Plate 1, Fig. 1*a*). The sutural lamellae are thick, sharp, and directed caudally. They, together, with the lamellae of the maxillary bone, form the maxillo-malar suture.

The malar bone penetrates deeply into the maxillary at the gomphosis and in cats often has in its anterior part a triangular fossa. When the fossa is present it is opposed by the small crest on the maxillary bone.

The lacrimal face of the maxillary bone together with the malar face of the lacrimal bone forms the rebated joint (sutura gradica) (Tucker 1954*c*). The maxillary region of the malar bone in the cat is much more curved (Plate 1, Fig. 3) than appears in the corresponding region of the dog. This condition is influenced by the great development of the optic bulbs. However, the influences of the eye bulbs cannot change the character and directions of transmitted stresses, which are due to the mechanical relations between the facial region and the neurocranium.

III. The Squamoso-malar Morphology

No less characteristic than the connections between the malar bone and the maxilla in Felidae are the connections between the malar bone and squamosal bone. The morphology of the sutural face of the squamosal process of the malar bone is similar to the internasal suturae. The sutural face is usually smooth. A special structure, the semisinus (a shallow depression in the bone which harbours the part of another bone) of the malar bone, is situated in the basal part of the frontal process of the malar bone (Fig. 2*a*; Plate 1, Fig. 3). The anterior part of the zygomatic process of the squamosal bone develops the appendix to the semisinus (Fig. 1*f*), a structure corresponding to the semisinus of the malar bone (Fig. 2; Plate 1, Fig. 3).

The semisinual appendix and the semisinus are specific structures for this type of zygomatic arch. The stresses are transmitted from the squamosal process of the malar bone towards the zygomatic process of the squamosal bone. In Canidae the connections between the malar and squamosal bones differ. However, there are some indications of structural similarities in the dog, even though the malar semisinus and the semisinual appendix are not developed.

In analysing the mechanics of these connections, the direction of stresses and the transmissive conditions must be considered. The stresses transmitted from the malar bone towards the squamosal bone do not provoke great changes in the sutural region because they are compressions. (What is more, this region functions similarly to the internasal junction.) The stresses transmitted from the squamosal bone to the malar bone are opposed in the suture by the action of the sutural lamellae. All other translocative tendency in this region is opposed by:

(1) The semisinus of the malar bone and the simisinual appendix of the squamosal bone; both structures together form a connection which has some similarity to the peg and socket connection.

(2) The gomphus and fossa gomphi as well as the rebated joint between the malar and lacrimal bones;

(3) The systems of lamellae which are situated in the neighbourhood of the external and the internal sutural walls of the maxilla.

The position of the semisinual process of the squamosal bone in the semisinus is assured by the maxillo-malar connections and the gomphus. The latter structures counteract the tendency of the malar bone to shift anteriorly. How-

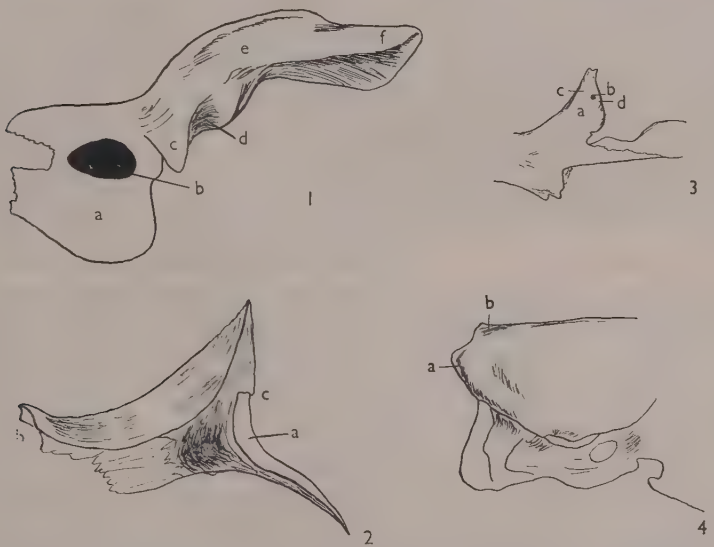


Fig. 1.—Lateral surface of the right zygomatic process of the squamosal bone and the appendix to the semisinus. *a*, Bulla ossea; *b*, meatus accusticus externus; *c*, postglenoid process; *d*, glenoid cavity; *e*, the zygomatic process of the squamosal bone; *f*, appendix to the semisinus.

Fig. 2.—The malar bone of the cat. Right bone, internal surface. *a*, Semisinus of the malar bone; *b*, anterior end of the malar bone; *c*, posterior end of the malar bone.

Fig. 3.—Foramen in the frontal process of the malar bone of the cat. *a*, Frontal process; *b*, foramen; *c*, anterior part of the frontal process; *d*, posterior part of the frontal process.

Fig. 4.—Shape of the occipital and frontal regions of the skull of Mustelidae, lateral view. *a*, Crista nuchalis; *b*, crista sagittalis.

ever, for as long as the anterior part of the malar bone remains in its position, all stresses in the body and frontal process of the malar bone are transmitted mainly towards the semisinual process of the squamosal bone. As a consequence of this, the squamosal bone transmits the stresses into the squamosal node, in spite of the absence of the sutural lamellae.

The direct transmission of stresses into the squamosal bone changes the relations in the zygomatic arch. The elongation of the ramus of the malar

bone increases stresses in the zygomatic arch, and this has an influence on the height and bending of the arch.

IV. The Connections of the Malar Bone

In Felidae the frontal process of the malar bone is often divided by the median fissure, which is situated between the foramen of the frontal process of the malar bone and the top of this process (Fig. 3). The median fissure has a tendency to close, and often is visible only as a median line. The line divides the frontal process into two uneven parts.

In Felidae, the abbreviation of the facial region is connected with the anterior translocation of the zygomatic arch. This causes:

(*a*) The approximation of the maxillary and premaxillary nodes. It is one of the expressions of a tendency towards the accumulation of stresses on limited spaces (Plate 1, Fig. 1).

(*b*) The formation of the premaxillo-supraorbital tract is greatly reduced.

The palato-maxillary region compared with the corresponding part in Canidae does not demonstrate distinct changes. However, in the sphenoccipital part of the cat skull the axis of the sphenoid and the long axis of the basi-occipitale are in nearly the same plane as the vomer. The basal angle of the skull, which is characteristic of the ruminants and rodents, is also demonstrated in Canidae.

The advanced and specialized changes in the described skull are expressed by:

(*a*) The further development of the zygomatic arch and the supraorbital node;

(*b*) The further development of the supraorbito-maxillary tract.

V. The Occipital Region

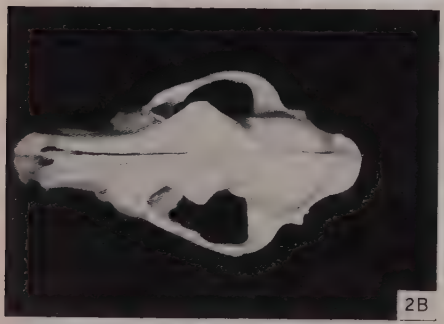
Although the main changes are observed in the premaxillo-supraorbital and maxillo-supraorbital tracts in the facial regions, the region of progressive accumulative changes in the neurocranium is in the interparieto-occipital node.

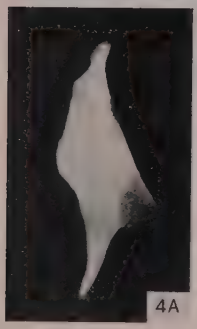
The only markedly developed structures in the posterior part of the skull in *Felis domestica* are the sagittal crest, nuchal crest, and the temporal crest of the squamosal bone. Being more strongly built than in the dog, the supraorbito-squamosal tract suggests the decreasing action of the masseter muscles in this region (relative to the temporal muscle).

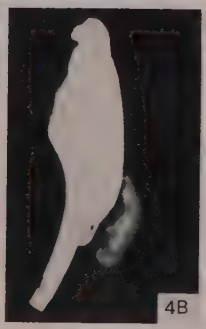
The tendency to diminution of the sagittal crest with a simultaneous tendency to enlargement of the nuchal crest is much better expressed in Mustelidae than in the cat. In *Mustela putorius* the rebated joint in the interparieto-occipito-squamosal tract and postprocessial sustentaculum are shaped in the same way as in the cat.

The nuchal crest has a tendency to enlargement and the interparieto-occipital node may even be square. The transformation from the triangular

FUNCTIONAL AND ANALYTICAL CRANIOLOGY. IV







to the square shape is distinctly visible on comparison of the skulls of the cat and Mustelidae as well as the skulls of the young and adult *Mustela* (Fig. 4).

This condition is quite common in many small Aeluroidea and probably is caused by the translocation of the endings of the temporal muscle as well as by the changes in the locomotion of these mammals.

In *Mustela putorius* the temporal muscle is developed to a high degree. The development of the dentition and the temporal (coronoid) process of the mandible gives evidence of this condition.

VI. References

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Explanation of Plates 1 and 2

Plate 1

Fig. 1.—The relation of the canine to the anterior border of the zygomatic arch in the dog and cat (lateral view, left side). *a*, The zygomatic line; *b*, posterior portion of the malar bone (the vertical black lines show the topographical relations of the canine and anterior border of the malar bone).

Fig. 2.—The distance between the postorbital processes filled by the postorbital ligament in the dog and in the cat. *A*, the cat; *B*, the dog.

Fig. 3.—The malar bone in the cat (*B*) (cf. Fig. 5). Internal side and interior view. *c*, Maxillary articular facet; *d*, curvature of malar bone; *e*, semisinus of the malar bone.

Fig. 4.—External face of the malar bone of the cat. In the middle part is visible the zygomatic line.

Fig. 5.—The internal side of the malar bone of the dog (*A*). *a*, Superior brachium of the malar bone; *b*, inferior brachium of the malar bone.

Fig. 6.—External face of the malar bone of the dog (*A*).

Plate 2

Fig. 1.—Maxilla of the cat. Lateral and medial sutural walls of the maxilla. The arrow shows the walls, and the triangular fossa between them.

Fig. 2.—Maxilla of the dog. The lateral sutural wall of the maxilla (in the maxillo-malar suture). The arrow shows the triangular fossa between the walls. *a*, Anterior end of the maxillary bone; *b*, lateral surface of the maxilla; *c*, superior surface of the alveolar process; *d*, palatine process of the maxillary bone; *e*, palatine bone; *f*, sphenopalatine foramen; *g*, posterior palatine foramen.

Fig. 3.—The lateral view of the maxillo-zygomatic connection in the dog. The arrows show the surfaces connected with the superior and inferior brachia of the malar bone.

STUDIES IN FUNCTIONAL AND ANALYTICAL CRANIOLOGY

V. THE FUNCTIONAL METAMORPHOSES OF THE CARNIVOROUS SKULL

By R. Tucker*

[*Manuscript received May* 31, 1954]

Summary

Four main positions of circumscribed stresses in carnivores are distinguished as exemplified by the conditions in (i) Canidae, (ii) Felidae, (iii) Machairondontinae and Odobenidae, and (iv) Otaridae and Phocidae. The functional transformations in the last two divisions are discussed with reference to the Canidae and Felidae. The changes in Phocidae and Otaridae are caused by the vanishing of the maxillary node. In *Smilodon,* the moments of the masseter and temporal muscle and the way in which the muscular torque is formed in the cat and *Smilodon,* are discussed. The extinction of *Smilodon* is explained in a mechanical way. In connection with it further relations of force are analysed with special reference to the torque and its moments. The skull of Mustelidae is briefly discussed as an example of transposition caused by a different type of locomotion.

I. The Scope of This Paper

In this paper will be considered only the transformations connected with changes in the position of the circumscribed stresses. These functional remodifications of the skull of carnivores have a far-reaching influence on the whole breviarcuate skull. The basic diet for all carnivores is the flesh and bones of animals for which, however, preparation for swallowing may be completely different. (The problem of relations between the skull's morphology and the diet will be discussed to a greater extent in one of the subsequent papers.)

As a standard position of stresses in the breviarcuate skull I shall use those in the dog and cat for they were functionally analysed in previous papers (Tucker 1954*c*, 1954*d*).

II. Position of Circumscribed Stresses in the Skulls of Carnivores

In Carnivora the following main combinations of the position of stresses can be distinguished:

(1) The greatest stresses are situated in the circumscribed region of the maxillary node. They are accompanied by a centrum of secondary importance (smaller stresses) in the premaxillary node at the level of the canines. This condition is present in Canidae (Fig. 1).

(2) The position of stresses basically the same as in (1), but with a strong tendency to shorten the premaxillo-maxillary tract and to increase

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stresses in the canine. This type is clearly developed in Felidae (Fig. 2).

(3) The still greater increase of stresses in the anterior part of the premaxillo-maxillary tract along the developmental lines of Felidae which, however, is now so great that it can hardly be considered of secondary importance. It is demonstrated in the Machairodontinae and in a different form in Odobenidae (Fig. 3).

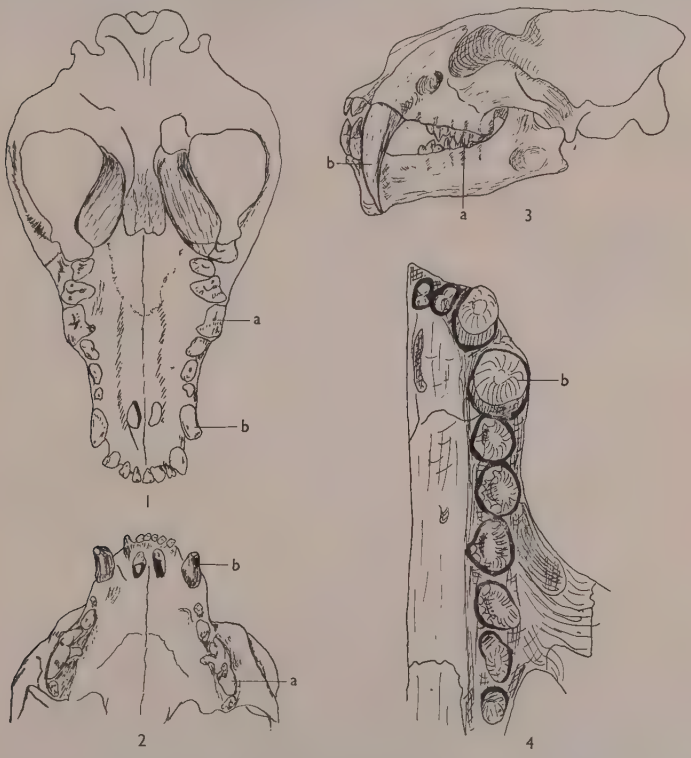


Fig. 1.—Position of circumscribed stresses in the dog (ventral view of the skull). *a*, The maxillary node; *b*, the canine.

Fig. 2.—Position of circumscribed stresses in the cat (ventral view of the skull). *a*, The maxillary node; *b*, the canine.

Fig. 3.—Position of circumscribed stresses in *Smilodon* (lateral view of the skull, left side) (after Abel). Explanations as Figures 1 and 2.

Fig. 4.—Position of circumscribed stresses in Otaridae (ventral view of the skull, left alveolar process) (after Wood Jones). *b*, The circumscribed stresses in the anterior part of the alveolar process.

(4) Only the anterior centrum of circumscribed stresses is present. The maxillary node is not developed at all. It is the case in Otaridae and Phocidae (Figs. 4, 5, 6).

It may be of interest to mention that the maxillary node is also not developed in Arctocyanidae (Creodontia: Ferrungulata), that is, in the very root of the phylogenetical tree of carnivores (Young 1950, p. 644).

The migration of the position of stresses is one of those questions in which the ecological and enological (Tucker 1953) problems meet; in other words, where the habitat of the ecological concept must be confronted with the internal relations of the organisms such as the question of construction, correlations, transformations, etc.

Fig. 5.—The skull of *Phoca leonina* (after Blainville). *b*, The canine.

III. Functional Transformations in the Skulls of Phocidae and Otaridae

In relation to the conditions in cats (and dogs) which were described previously (Tucker 1954*c*, 1954*d*) the fronto-malar connections are rather weak. The postorbital process may be short as in *Arctocephalus* (Otaridae) or vanish completely as in *Leptonychotes* or *Hydrurga* (Phocidae). Further transformations which correspond to these are present in the frontal process of the malar bone.

Finally, in association with the vanishing of the maxillary node, the skull loses its breviarcuate character. The principal cranial arch is not demonstrable in either of its parts—the postorbito-squamosal (temporal) and postorbito-maxillary tracts. The whole skull is markedly flat. In *Arctocephalus cinereus* (Otaridae) the zygomatic arch is still bent up while in *Leptonychotes* and *Hydrurga* (Phocidae) it is completely flat. (The transformations in the mammalian skull due to the migration of nodes will be discussed in greater detail in subsequent papers.)

IV. Changes in Cranium of Odobenidae and Machairodontinae

The development of the facial part of *Trichechus* (Odobenidae) (Fig. 6), which is an earth digger, is very great. The maxillary node, as in Phocidae and Otaridae, vanishes completely. The premaxillary node is rudimentary and the incisors are absent at the anteroventral margin of the premaxillary bone. They are pushed back towards the maxillary bone. There is a marked shortening of the nasal bones. The maxilla is very large and the fronto-premaxillary connections are absent. The zygomatic arch is again flat (Fig. 6), while the squamosal part of the occipital bone, and nuchal crest, are prominent. The sagittal crest is not developed at all.

Fig. 6.—Skull of *Trichechus rosmarus* (after Blainville). *a*, Maxilla; *b*, canine; *c*, premaxilla; *d*, zygomatic arch; *e*, nuchal crest and interparietal bone.

The cranium of *Smilodon* (Machairodontinae: Felidae) (Fig. 3) is different. It is a typical breviarcuate skull. The shortening of the premaxillo-maxillary tract is also pronounced. The point of interest for functional investigations is that *Smilodon* used the whole head for killing prey and accordingly exerted, in this action, the stresses originated from the cervical and not from the masticatory muscles. The position of the masseter, temporal, and digastric muscles as well as the cleido- and sterno-mastoid muscles in *Smilodon* and *Felis* was studied by Matthew (Young 1950, p. 659). The reconstructions of Matthew will form the basis for the present considerations. The greater development of the cleido- and sterno-mastoid muscles in *Smilodon* in regard to the function of its head is understandable, as is also their contribution to the development of the squamosal part of the occipital bone. On the other hand, the forces which originate from these muscles contribute to the formation of the interparieto-occipital node and to the shaping of the occipito-squamosal junction.

But from the aspect of functional analysis the main points of interest are:

(*a*) The longer, toothless margin of the mandible in *Smilodon* than in the cat;

(*b*) The narrower ramus of the mandible in *Smilodon* than those in the cat;

(*c*) The less developed fronto-zygomatic connections in *Smilodon* than those in the cat;

(*d*) The relatively narrower but longer temporal muscle in *Smilodon* than in the cat;

(*e*) The narrower but longer masseter in *Smilodon* than in the cat;

(*f*) The greater distances travelled by the mandibular incisors when the mouth is closed in *Smilodon* than in the cat.

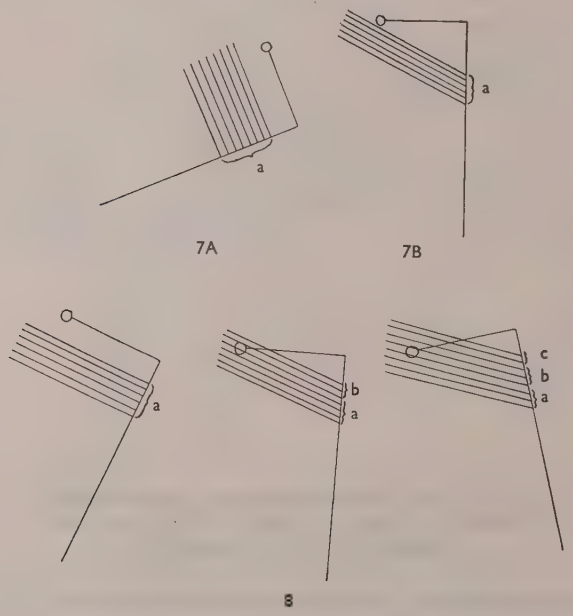
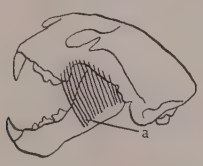


Fig. 7.—Diagram of the masseter in cat (*A*) and *Smilodon* (*B*). *a*, Functionally effective part of the muscle. For orientation compare with Figure 9.

Fig. 8.—The position of the mandibular insertions of masseter by various locations of the mandible of *Smilodon*. *a*, Functionally effective part of the muscle; *b*, the fibres with zero moment; *c*, the fibres with negative moment. For orientation of masseter compare with Figure 9.

In mechanical terms this means that the initial stages of the muscular contraction require much more force and energy in *Smilodon* than in cats. Accordingly, the masticatory and killing system in cats is more economic—a smaller number of muscles is involved. Their masseter and especially their

temporal muscle are in a better functional position. The masseter of *Smilodon* is bound to be small because by the opening of the mandible, its posterior part is functionally useless. It is demonstrated in Figures 7, 8, and 9. The masseteric fibres situated at the level of the glenoid cavity in the fully open mouth have a moment equal to zero when the fibres situated in the same movement behind the glenoid cavity have a negative moment (Figs. 8, 9). Therefore in the second case the force exerted by the muscle has no functional use, whereas in the third it would prevent the closing of the jaw. But the consequent anterior shifting of the zygomatic insertions of the masseter in reference to the



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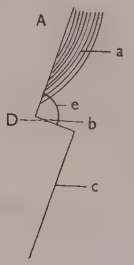

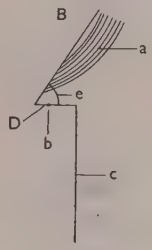

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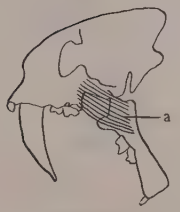

9B

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Fig. 9.—The outlines of the skulls of cat (*A*) and *Smilodon* (*B*) with the masseter shown. This diagram should be compared with Figures 7 and 8 for orientation of the masseter which in Figures 7 and 8 is demonstrated schematically. *a*, Masseter.

Fig. 10.—The mandibulo-temporal relations in the cat (*A*) and *Smilodon* (*B*). *a*, Temporal muscle; *b*, cranio-mandibular joint; *c*, mandible; *d*, coronoid process; *e*, angle between the coronoid process and the axis of the temporal muscle during the movement of the lower jaw. For orientation compare with Figure 12.

Fig. 11.—Position of fibres of temporal muscle in *Smilodon* (*A*) and cat (*B*). For orientation compare with Figure 12.

whole structure of the zygomatic arch causes changes in the morphology of the arch. The free posterior part of the zygomatic arch has moved down in a way resembling a like effect in the longoarcuate skull, which will be analysed in detail later. The transformations of the zygomatic arch in turn influence the fronto-zygomatic connections which in *Smilodon* resemble the condition in dogs more than that in other cats. Among numerous other changes two seem worth mentioning—these are the differences in development of the interparieto-occipital node and the anterior part of the alveolar process of the mandible. In *Smilodon* the anterior part of the alveolar process of the mandible is unde-

veloped, the incisors being small and the adental margin very long. This is again the expression of unfavourable mandibulo-temporal relations in *Smilodon* (Figs. 10 and 12) which cause a great loss of muscular strength and in which the moment of the temporal muscle presents a greater accumulation of stresses on the incisor or on the site of the usual position of the mammalian premolars.

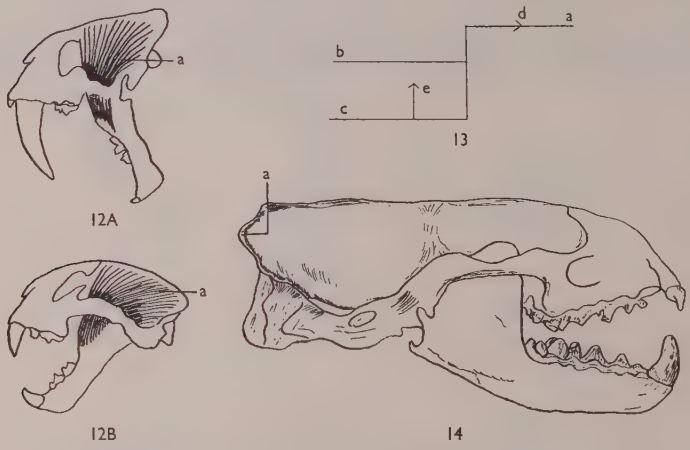

Fig. 12.—The outlines of the skulls of *Smilodon* (*A*) and the cat (*B*) with the temporal muscle shown. This diagram should be compared with Figures 10, 11, or 13 for orientation of the temporal muscle which in those figures is demonstrated schematically. *a,* Temporal muscle.

Fig. 13.—Functional relation of the horizontal fibres of the temporal muscle to the closing of mandible and tightening of the grip. For orientation compare with Figure 12. *a,* Horizontal fibres of the temporal muscle; *b,* maxilla; *c,* mandible; *d,* vector during contraction of the horizontal fibres of the temporal muscle; *e,* vector in mandible resulting from the vector *d.*

Fig. 14.—Position of nuchal crests in the *Mustela* (after Blainville). *a,* Nuchal crest.

The interparieto-occipital node in *Smilodon* differs in shape from that of a cat because the angle at which the supraorbito-interparieto-occipital and squamoso-interparieto-occipital tracts meet is more acute in *Smilodon* than in the cat. It is accompanied by differences in the direction of the fibres of the temporal muscle (after Matthew from Young 1950, p. 650). This difference is demonstrated in Figures 11 and 12. Consequently the interparieto-occipital node in *Smilodon* under the influence of the temporal muscle develops more accumulated circumscribed stresses than in the cat. Still another consequence of this arrangement is that the horizontal fibres of the temporal muscle are the parts which tighten the grip (Figs. 12 and 13). Consequently the grip of *Smilodon* was rather weak (also compare with previously mentioned morphology of the anterior part of the mandible).

V. Moment of the Masseter

So the main differences in the skulls of *Smilodon* and cat can be traced to the moment of the masseter. Moreover the unfavourable moment of the masseter became the direct cause of the extinction of the species.

Usually it is stated that these animals probably fed on large, thick-skinned herbivores and disappeared when their prey became rare (Young 1950, p. 650). This is a rather general and insufficient accounting and the rather common acceptance of it is an example of how easily we tend to grasp the first apparent explanation. In the light of functional analysis the problem may appear as follows: *Smilodon* could kill only large animals. The method of killing by striking with the head and very large canines requires a sufficiently large surface. While the head was lifting, the *Smilodon* lost sight of the victim, but in a large animal the burying of the teeth into the flesh was effective immaterially of whether it struck 12 in. more anteriorly or posteriorly. It is quite different when the prey is relatively small; it means that the prey is lost. In this way it would certainly be impossible to kill a mouse. The key to the problem is by the above discussed analysis of the masticatory system. Generally, carnivores can vary their food more easily than herbivores and there are numerous examples of it. But *Smilodon* could not change. The movements of its jaws are not suitable for catching prey. This results from the unfavourable moment of the masseter and probably of the temporal muscle also—not external lack of meat but internal inability to change the grip. Dogs and modern cats are much more versatile in their masticatory apparatus and much more economic.

The comparison of the conditions of the muscular contractions in *Smilodon* and the cat forms a good illustration of the relations between force and position. It has been established that

$$F \quad r \quad PTc \quad MPc \quad TPm \quad Mpm$$

(Tucker 1954*a*) (this giving the length of the muscle).

Because the relations of the cranial and mandibular insertions of both muscles influence and consequently define the torque in the sense demonstrated in Figure 7, these relations could be written as:

$$F \quad r \quad \frac{TPc}{TPm} \quad \text{and} \quad \frac{MPc}{MPm}$$

(This defines the angle between the bar passing through *b* and the muscle *a* in Figure 7.)

However, the final element of this chain is created by relations between the mandibular insertions of the masseter and temporal muscles to the length of the mandibular lever so formed. This defines the moment of force. Consequently

$$P^{II} = \frac{MPm}{m1} \quad \text{and} \quad \frac{TPm}{m1}$$

when P^{II} is the mandibular insertions of both muscles and $m1$ the length of the mandible. Then from

$$F \quad r \quad PTPc \quad MPc \quad TPm \quad MPm$$

can be written

$$F \quad r \quad PP^{I} \quad P^{II},$$

when

$$P^{I} = TPe \quad MPe \quad TPe \quad TPm \quad MPm,$$

and

$$P^{II} = \frac{MPm}{m1} \quad \text{and} \quad \frac{TPm}{m1}.$$

The relation of $\frac{P}{P^{II}}$ defines the given moment and so $F \quad r \quad \frac{P}{P^{II}}$ and sometimes may be the cause of death or survival.

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STUDIES IN FUNCTIONAL AND ANALYTICAL CRANIOLOGY

VI. STRAINS AND THE DIRECTION OF CERTAIN VECTORS IN THE BREVIARCUATE SKULL

By R. Tucker*

[*Manuscript received May* 31, 1954]

Summary

The strains in the anterior and posterior positions in the breviarcuate skull are illustrated by means of fractures. The vectors in the anterior triangle of the hyena skull are demonstrated by means of the dental attrition and compared with those of the leopard.

I. The Functional Masticatory Asymmetries

The animals of prey when masticating hard tissues, especially bones, rather often operate one side of the skull and mandible only. One end of the bone is placed on one carnassial only. Similar functional asymmetries occur in the anterior position of the accumulated stresses during attack or combat. Accordingly, the unusually great and suddenly rising stresses† can become great enough to be destructive. These stresses should be of a special interest from the functional approach, especially in the breviarcuate skull where the accumulation of stresses is so well pronounced.

II. Strains in the Anterior Position

Colyer (1936) in his extensive survey of the dental abnormalities and deformities in animals, reported a few cases of fractures which may illustrate certain points of the functional analysis (Tucker 1954*a*, 1954*b*, 1954*c*, 1954*d*, 1954*e*). Colyer's approach is not functional. He does not think of any causes other than the external ones. The interpretation is, however, something quite different from the specimens and, as opposed to the demonstrated structures, can always be changed. Accordingly, this paper is offered as a functional interpretation.

Figure 1 demonstrates one of Colyer's cases. It is a lion with a fractured left maxilla. *The canine is broken off.* Sudden and asymmetrical rise of stress in the anterior position injured the canine and caused, in agreement with previous analysis (Tucker 1954*a*, 1954*b*, 1954*c*, 1954*d*, 1954*e*) an equally sudden increase of strains in the premaxillo-maxillary and premaxillo-supraorbital tracts.

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† The musculature of both sides is acting simultaneously. It can be checked on anybody's mandible by pressing the fingers against the left and right masseter when crushing food with one side of dentition only.

This results in the breaking off of the premaxillo-maxillary tract just before the maxillary node. The strain-fracture is roughly parallel to the canine alveolus.

The translocation of the anterior part of the maxilla is accompanied by fractures in the region of the frontal process of the maxillary bone (Figs. 1, 2). Both these fractures are a result of rotatory tendencies in the maxillary bone, especially after disjunction of the premaxillo-maxillary tract.

Fig. 1.—The skull of lion (after Colyer 1936), showing the fractured maxilla.

Fig. 2.—The skull of lion (after Colyer 1936), showing the fractures in nasal bones (*a*) and in the frontal process (*b*) of maxilla.

Fig. 3.—The fractured mandible of cheetah (after Colyer 1936).

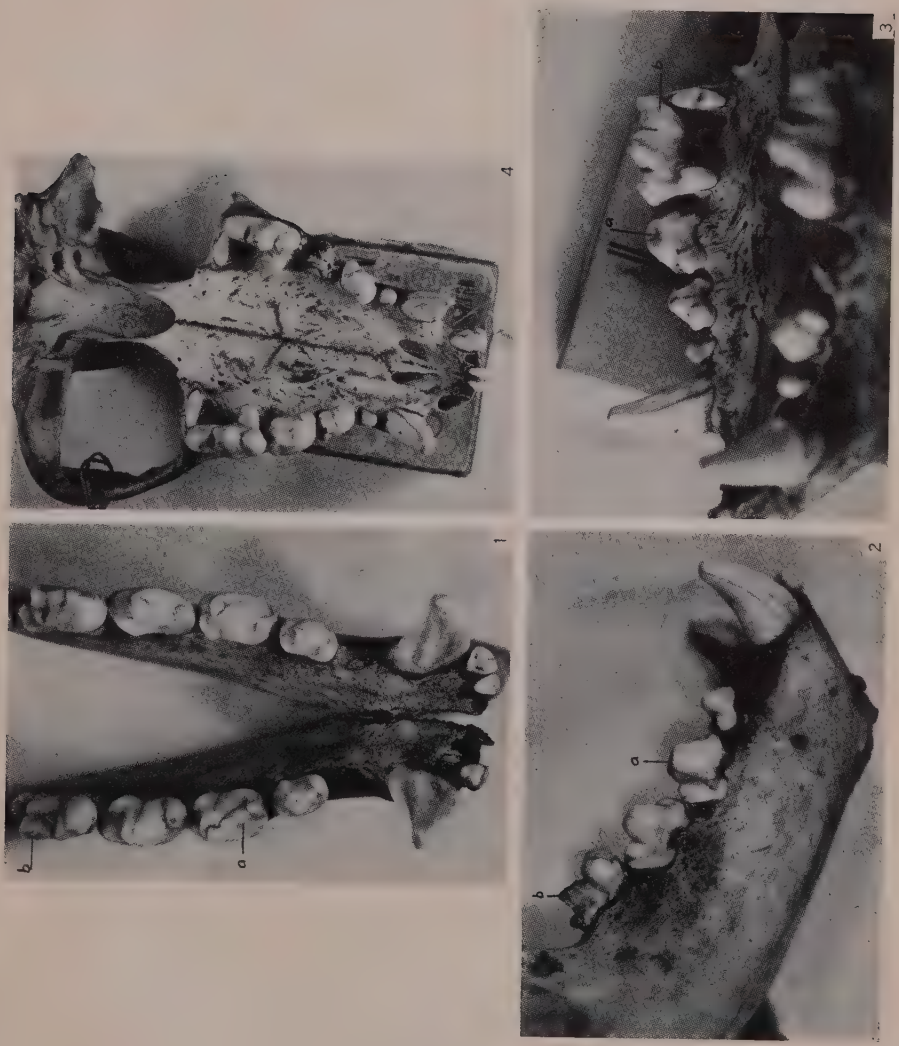
Fig. 4.—The fractured mandible of leopard (after Colyer 1936).

Fig. 5.—The axes of the teeth in leopard (after Colyer).

However, in the anterior triangle of the breviarcuate skull there exist, as well as the rotatory tendencies, transverse stresses. These, in cases of even slight lateral translocation, increase rapidly at the upper part of the structure at the level of the nasal bones (Tucker 1954*c*). In the described case (Fig. 2) the nasal bones are distorted and their anterior parts translocated towards the opposite side (to the right).

The locations of all these areas of fractures corresponds closely to the regions of excess strains which originate from the force exerted in its anterior position (premaxillary node) (Tucker 1954*a*).

FUNCTIONAL AND ANALYTICAL CRANIOLOGY. VI



III. Strains in the Posterior Position

In Colyer's collection is a specimen of cheetah with a fractured mandible, i.e. in the position of posterior strains (Fig. 3). The mandible is broken at the level of the first premolar, which functionally corresponds to the carnassial (*P4*). Colyer (1936) remarked about this specimen that "the impact of the blow causing the injury would appear to have been received on the first molar, as the anterior cusp is broken and the posterior cusp splintered slightly" (pp. 491-2). It may be added that "the blow" was placed in the main position of accumulated strains in the breviarcuate skull, between the carnassial and the first mandibular molar and in agreement with the direction of transverse strains which in the mandible has the opposite direction to those of the maxilla.

Another example is given of the fracture of the mandible in a leopard (Fig. 4), also in the region of the maxillary node (fourth premolar).

IV. Direction of Vectors in the Hyena

The effect and especially the direction of stresses can also be conveniently studied on the location of dental attrition. In the Queensland Museum is a specimen of the skull of a hyena with very distinct attrition in nearly all teeth (No. g. 6672). In the hyena the mandibular premolars occupy a rather external position, and the row of dentition is usually not so strongly bent as in Canidae. This results in more close superposition of upper and lower rows of dentition. Accordingly, the most prominent transverse stresses are at the level of the maxillary node.

In the mandible all premolars demonstrate attrition right at the top of the dental cusps (Fig. 5) while the molars show lateral attrition on the external side of the teeth (Fig. 5; Plate 1, Fig. 2).

In the maxilla, first, second, and third premolars expose attrition at the top of the cusps, whereas the fourth premolar has medial attrition (Plate 1, Figs. 3 and 4).

The relations between the long axis of the maxillary teeth and the long axis of the skull are very characteristic (Plate 1, Fig. 4), the fourth premolar being the single tooth whose long axis is parallel to the interpalatine and intermaxillary sutures. The transposition of the axes of the first, second, and third maxillary premolars with regard to the long axis of the fourth premolar, causes a decrease of the transverse stresses in the anterior triangle. The dental attrition in hyenas shown in the photographs of Colyer (1936) is of the same type.

Figure 5 shows the axes of the premolars in the maxilla of the leopard. This figure when compared with the hyena shows the difference in the magnitude of the transverse stresses in these two animals.

V. Acknowledgment

I wish to thank Mr. G. Mack, Director of the Queensland Museum, for providing ready access to the specimens.

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Explanation of Plate 1

Fig. 1.—Attrition in the mandibular dentition of hyena. *a*, Attrition at the top of cusp; *b*, lateral attrition.

Fig. 2.—Attrition of the mandibular dentition in hyena.

Fig. 3.—Attrition in the maxillar dentition of hyena. *a*, Attrition at the top of cusp; *b*, medial attrition.

Fig. 4.—The skull of hyena. Ventral view. The axes of the teeth.

THE CLASSIFICATION AND DISTRIBUTION OF TABANIDAE (DIPTERA)

I. GENERAL REVIEW

By I. M. MACKERRAS*

[*Manuscript received May* 17, 1954]

Summary

The old primary classification of the Tabanidae, based on the presence or absence of hind tibial spurs, has been replaced by one based primarily on the genitalia of both sexes, but supported by a significant, though not complete, correlation with external characters. The early stages, so far as known, support the new arrangement. The following subfamilies and tribes are recognized:

Pangoniinae	*Scepsidinae*	*Chrysopinae*	*Tabaninae*
Pangoniini		Bouvieromyiini	Diachlorini
Scionini		Chrysopini	Haematopotini
Philolichini		Rhinomyzini	Tabanini

Distribution of the family is world-wide, but can be divided into three main sections. More primitive groups are predominantly southern, occurring in some or all of South America, southern Africa, Australia, and New Zealand, with occasional northern extensions, and, in one case (Pangoniini), an extensive Holarctic arc. More specialized groups show two patterns. Southern regional radiations have occurred in South America and Africa, with extensions respectively into the Nearctic and Oriental-Australasian regions. Northern radiation of Chrysopini, Haematopotini, and Tabanini has resulted in a radial distribution like that of the eutherian mammals.

I. INTRODUCTION

If a group is to be used in zoogeographical studies, the first essential is that its classification should present a true picture of its broad evolutionary history and of the relationships between its members. No one today is fully satisfied with existing classifications of the Tabanidae, and, with the single exception of the well-defined genus *Scaptia*, it is doubtful whether anyone would be willing to use members of this family in zoogeographical reasoning. My own dissatisfaction began with the primary subdivision, which placed the chrysopines unhappily with the pangoniines, and separated them from the tabanines, to which they seemed to me to be much more closely related. It appeared that new characters should be sought, and those that had already been used, re-appraised.

External characters had been thoroughly examined by previous workers, but the terminalia had been largely neglected, and it therefore seemed desirable to make a careful survey of these parts. The results were extremely inter-

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esting, the family falling readily into major groups on characters of one or both sexes, with well-defined differences between the groups, and remarkable general uniformity within them. The groups appear to be natural, in that the genitalic characters rarely tend to merge into one another, there is no evidence of parallel evolution in them, there is good general correlation between genitalic and external characters, and the little that is known of the larvae and pupae fits the classification based on the terminalia much better than the old arrangement based on the hind tibial spurs (Hennig 1952).

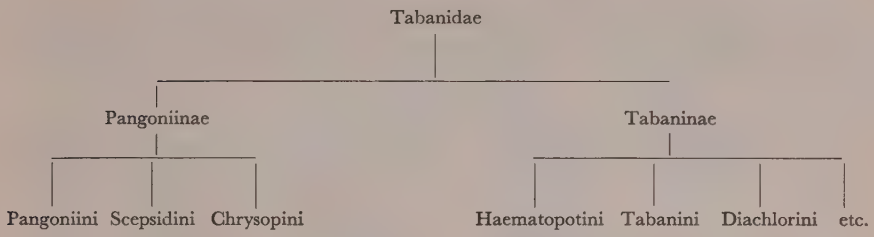
The chief objections that have been raised to the use of terminalia are, that dissection is tedious and requires manual dexterity, that some mutilation of the specimens is inevitable, and that the characters found are sometimes confined to one sex. The first two, I feel, must be faced, when hypopygial differences have been shown to have real value — we must take our characters where we find them. The third is more important, because one would expect differences to have developed in both sexes, in groups which have been reproductively isolated from one another for the long geological periods that must have elapsed since the ancestors of existing subfamilies and tribes diverged. Single characters should, indeed, be used with caution at the supra-generic level, but in a relatively uniform, difficult family like Tabanidae, any reasonably stable character is worth considering.

The classification will be examined in detail in a later paper. It is proposed here to treat it broadly, giving simply an outline down to tribes, and citing only sufficient characters to define the groups, with enough illustrations to indicate the nature of the differences described. Genera, with few exceptions, will not be discussed at the present stage.

It is necessary to state that the classification was developed on purely morphological evidence. Zoogeographical consequences were not considered, and some of them, particularly the extent of the African relationships with South America and Australia, came as a complete surprise. From the systematic point of view, distribution is an important criterion in assessing species and subspecies, but its only value at higher levels is when an unusual distribution makes us re-examine the validity of the characters we have used. Enderlein's badly founded genus *Phyrta*, with a distribution in China-Japan-Indonesia on one side of the world and South America on the other, is a case in point.

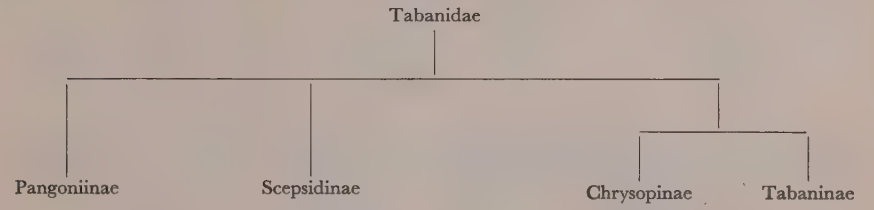
II. Classification

Loew, in 1860, divided the Tabanidae into two subfamilies, Pangoniinae and Tabaninae, on the presence or absence respectively of paired spurs on the hind tibiae. Lutz, in 1909, raised these divisions to sectional rank in order to provide for an array of subfamilies under each. Enderlein (1922, 1925) based his elaborate classification on Lutz's divisions, but modern workers (e.g. Bequaert 1930; Fairchild 1942; Philip 1947, 1950) have tended to return to the old subfamilies, with a series of tribes in each, the arrangement being somewhat as follows:



The objections to this classification are: firstly, that the hind tibial spurs are variable, and tend to disappear in some pangoniines and chrysopines; and secondly, as already stated, that it gives a false impression of relationships.

The classification here proposed is shown in the following diagram, the further subdivision of the major subfamilies being omitted for simplicity.



While convenience cannot be considered in searching for characters, classifications at best are imperfect things, and convenience for workers in the group does merit consideration, provided that it does not obscure relationships. The present classification ranges the subfamilies in a broad evolutionary sequence; but it does not show, without reference to the diagram, that the Chrysopinae and Tabaninae are more closely related to each other than to the preceding groups. Similarly, the tribes recognized do express broad natural relationships, and are convenient for the general worker, particularly in zoogeography; but they do not represent equal ages or equal degrees of evolutionary divergence. The tribes of Pangoniinae are certainly the most ancient, those of Chrysopinae probably intermediate, and of Tabaninae most recent.

Correlated with age are varying degrees of precision with which the tribes can be differentiated, and this affects their usefulness to the systematic worker. In the Pangoniinae, the distinctions appear to be precise, although dissection is essential for accurate determination. Differentiation in the Chrysopinae is also fairly good, but there is a tendency for Bouvieromyiini and Chrysopini to merge, and the Rhinomyzini may not be entirely monophyletic. The Tabaninae are a different story. It is clear that a considerable number of separate groups have evolved from primitive Diachlorini. Of these, the Haematopotini in the north are precisely differentiated. The Tabanini, also a predominantly northern group, have been extremely successful, with very many species, the vast majority of which can be clearly placed, leaving a comparatively small transitional

series of intermediates. The position is further complicated, however, by evidence suggesting that diachlorine types may still be evolving tabanine characters in South America.

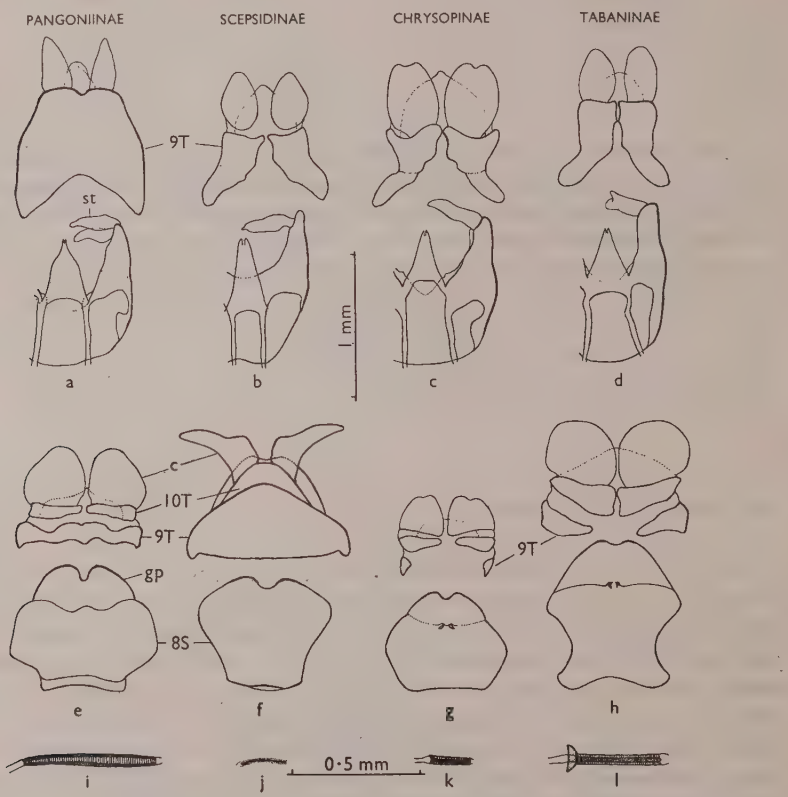


Fig. 1.—Hypopygial characters of subfamilies. *a-d*, Males; *e-h*, females; *i-l*, caudal ends of spermathecal ducts of females. *a*, *Pangonius mauretanus* (Linn.), N. Africa; *b*, *Adersia oestroides* (Karsch), Africa; *c*, *Veprius presbiter* Rond., Chile; *d*, *Hybomitra rhombica* (O.-S.), N. America; *e*, *Pangonius mauretanus* var. *aethiops* (Szil.), N. Africa; *f*, *Scepsis nivalis* Walk., S. America; *g*, *Silvius vituli* (Fabr.), Europe; *h*, *Tabanus bovinus* Linn., Europe; *i*, *Scaptia patula* (Walk.), Australia; *j*, *Scepsis nivalis*; *k*, *Silvius vituli*; *l*, *Tabanus bovinus*. *st*, *Style*; 9*T*, ninth tergite; 10*T*, tenth tergite; *c*, cerci; 8*S*, eighth sternite of ♀; *gp*, anterior gonopophyses of ♀.

We are left with a number of rather highly specialized groups, which evidently developed independently from diachlorine ancestors. There are at least five of these in tropical South America and two in the Oriental and Australasian regions. It could be claimed that they are as well defined morphologically as

the tribes already recognized. However, their differentiation depends largely on the development of curious and bizarre characteristics, for which there is a parallel in another tropical group, the Rhinomyzini; five of them contain only a single genus; and to recognize 10 or possibly more tribes in the subfamily seems under the circumstances to be over-working a useful category. I have therefore left them as "specialized groups" within the Diachlorini.

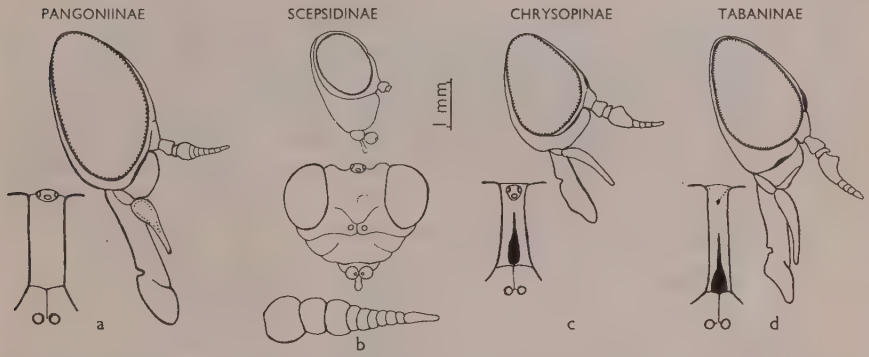


Fig. 2.—Heads and frons of generalized representatives of subfamilies. *a, Scaptia aurata* (Macq.), Australia; *b, Scepsis nivalis* (antenna from a drawing by Mr. H. Oldroyd at a higher magnification); *c, Veprius presbiter*; *d, Cydistomyia postica* (Wied.), Australia.

Subfamily PANGONIINAE

Ninth tergite undivided, a large chitinized shield in male, a transverse bar in female (Fig. 1). Antennae short (Figs. 2, 4, 5, 7), third segment nearly always 6-8-annulate. Proboscis and mouth-parts well developed, sometimes exceedingly long. Hind tibiae nearly always with paired apical spurs. Vein *sc* bare above and below. Caudal ends of spermathecal ducts of female simple tubes without mushroom-like expansions (with a lateral projection in *Mycteromyia*).

Tribe PANGONIINI

Style of male hypopygium bifid (Figs. 1, 3). Eighth sternite of female with gonopophyses close together (Figs. 1, 3*d*). Eyes usually bare. Ocelli always present. Callus sometimes developed. Antennae with basal section of 3rd segment often swollen, and the basal 4 annuli sometimes more or less completely fused, leaving an apical portion of 4, or rarely 3, annuli (Fig. 4). Palpi variable, sometimes small and with lateral concavity, sometimes larger and subcylindrical or sabre-shaped. Vein R_4 nearly always with strong appendix.

*Genera examined.**—These fall into two groups:

More generalized—cell R_5 open; proboscis relatively short, labella large:

* Genera are listed under existing names, without reference to changes and reductions that may be necessary; subgenera are listed as if they were genera. Those of which the genotypes have been examined are printed in small capitals.

PILIMAS, STONEMYIA, BRENNANIA, APATOLESTES, ASAPHOMYIA, CHAETOPALPUS, PARASILVIUS, ECTENOPSIS, CAENOPROSOPON, and two unnamed Australian genera. (*Protodasyapha* can also be placed here from Hack's (1953) figure of the male hypopygium.)

More specialized—cell R_5 closed; proboscis relatively long and slender, labella unexpanded, chitinized: PANGONIUS, *Esenbeckia*, PROBOSCOIDES.

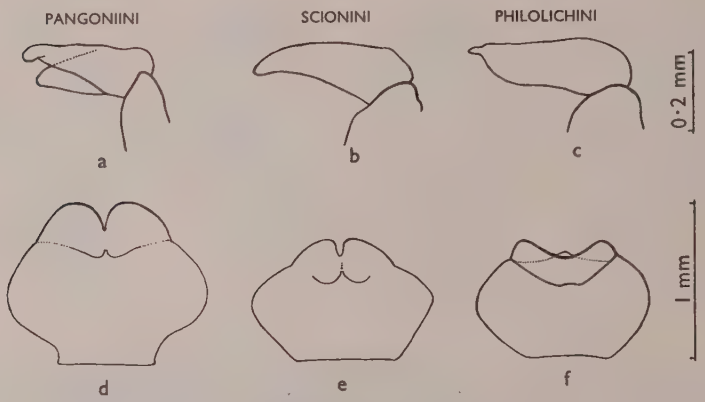


Fig. 3.—Pangoniinae. Hypopygial characters of the three tribes. *a-c*, Styles of males; *d-f*, eighth sternite and anterior gonopophyses of females. *a*, *Apatolestes comastes willistoni* (Bren.), N. America; *b*, *Fidena sorbens* (Wied.), S. America; *c*, *Buplex ?albifacies* (Ric.), S. Africa; *d*, *Pilimas californica* (Big.), N. America; *e*, *Scaptia barbata* (Linn.), S. Africa; *f*, *Buplex suavus* (Loew), S. Africa.

Tribe SCIONINI

Style of male hypopygium simple, finger-like, rarely pointed (Fig. 3*b*). Eighth sternite of female normal. Eyes nearly always hairy. Ocelli well developed. Third antennal segment subulate, 7- or 8-annulate. Palpi nearly always flattened, and usually with a bare lateral concavity. Vein R_4 usually without or with only a small appendix.

Genera examined.—GONIOPS, MYCTEROMYIA, *Fidena*, *Melpia*, *Scione*, PITYOCERA, *Listriosca*, PAROSCA, LISTRAPHA, OSCA, SCAPTIA, PALIMMECOMYIA.

Tribe PHILOLICHINI, trib. nov.

Style of male hypopygium simple, pointed, usually wider than in Scionini. Distal edge of 8th sternite of female forming a characteristic concave chitinized projection, with the anterior gonopophyses widely separated (Fig. 3*f*). Eyes bare. Ocelli absent, except in *Buplex*. Antennae as in Scionini. Palpi nearly always small, flattened, with or without bare lateral concavity. Vein R_4 with strong appendix.

Genera examined.—BUPLEX, *Ommatiosteres*, PHILOLICHE, METAPHARA, NUCERIA, *Stenophara*, DORCALOEMUS, *Phara*. (*Subpangonia* also appears to belong to this tribe.)

Subfamily SCEPSIDINAE

Ninth tergite divided, chrysopine-like, in male; a single large triangular arched plate in female (Fig. 1). Eyes bare. Ocelli well developed. Antennae short, pangoniine-like; 3rd segment more or less subulate, 6-8-annulate. Proboscis extremely short and weak, probably non-functional; palpi very short, both segments more or less bulbous (Fig. 2). Hind tibiae with paired apical spurs. Vein *sc* bare above and below. Style of male hypopygium single, pointed, chrysopine-like. Eighth sternite of female also rather chrysopine-like, but 9*T*, 10*T*, and cerci quite distinctive (Fig. 1). Caudal ends of spermathecal ducts slender, unexpanded tubes.

Genera examined.—SCEPSIS, ADERSIA. (Others are *Braunsiomyia* and *Lesneus*.)

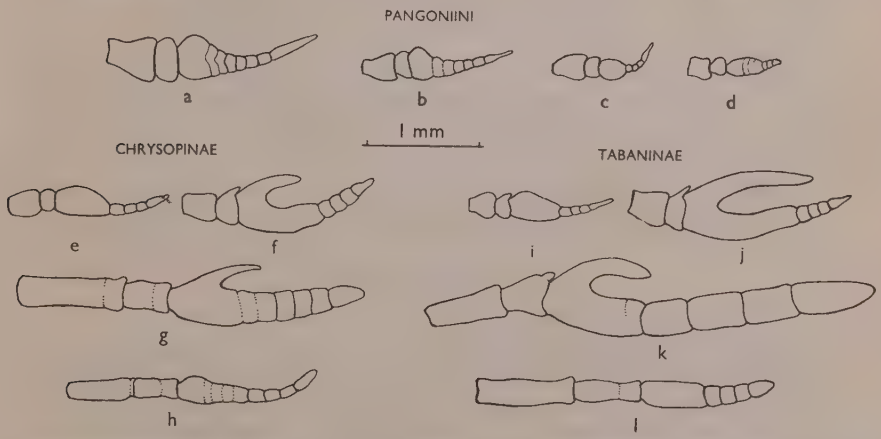


Fig. 4.—Antennae of females. Top row: Pangoniini, showing progressive reduction of annuli of third segment. *a*, *"Corizoneura" chrysophilus* (Walk.), Australia; *b*, *Parasilvius fulvus* Ferg., Australia; *c*, *Chaetopalpus coracinus* Phil., Chile; *d*, Gen. nov., sp. nov., Western Australia.

Remaining rows: illustrating parallel development. Chrysopinae: *e*, *Mesomyia montanus* (Ric.), Australia (generalized); *f*, *Tabanocella denticornis* (Wied.), Africa; *g*, *Gastroxides ater* Saund., India; *h*, *Chrysops caecutiens* (Linn.), Europe. Tabaninae: *i*, *Leptotabanus transversus* (Walk.), New Zealand (generalized); *j*, *Psalidia fulminea* (Hine), S. America; *k*, *Acanthocera apicalis* Fchld., S. America; *l*, *Udenocera brunnea* Ric., India.

Subfamily CHRYSOPINAE

Ninth tergite divided; a pair of large, characteristically shaped plates in male*; small, widely separated, triangular plates in female (Fig. 1). Antennae short or long (Fig. 4); 3rd segment with the basal 4 annuli more or less wid-

* Narrowly united in mid line in *Aegophagomyia* and a few species of *Mesomyia*.

R_4 usually with strong appendix. Typically slender species, usually with characteristically dappled wings.

Genera examined.—HAEMATOPOTA, HEPTATOMA, HIPPOCENTRUM.

Tribe TABANINI

Basicosta strongly setulose, except in 6 Palaearctic species. Frons medium to narrow, usually converging. Antennae short and usually stout; 3rd segment nearly always with well-developed dorsal angle, sometimes strongly hooked, apical portion almost always with 4 annuli. Labella large and soft, very rarely with a narrow chitinous bar. Wing sometimes infuscate or pictured, but never finely dappled.

Genera examined.—TABANUS, THERIOPLECTES, STYPORHAMPHIS, HYBOMITRA, ATYLOTUS, ABATYLOTUS, *Phyrta,* DASYRHAMPHIS, EFFLATOUNANUS, *Glaucops,* AGKISTROCERUS, HAMATABANUS, WHITNEYOMYIA, LEUCOTABANUS, *Myiotabanus,* LOPHOTABANUS, PHILIPOTABANUS, *Taeniotabanus,* EUANCALA, *Ancala.*

Note: *Dasyrhamphis* and two species of *Tabanus* with bare basicosta are felt to be properly included; *Efflatounanus,* also with bare basicosta, and *Glaucops,* with only a few setulae, show some Diachlorine features, and are included provisionally.

III. Evolution and Distribution

In the absence of fossils earlier than the Tertiary, any account of the evolution of Tabanidae must necessarily be speculative, and based primarily on the comparative morphology of recent Tabanoidea, and secondarily on distributional parallels with other groups whose geological history is better known. Nevertheless, an indication of probable evolutionary history seems desirable as a background to the zoogeographical facts to be presented.

There appear to be three roughly fixed points. Tillyard (1935) placed the origin of the Diptera in the Permian. From various statements, such as those by Walkom (1949) and Mayr *et al.* (1952), it may be inferred that groups with a Gondwanaland distribution must have evolved not later than the middle of the Mesozoic; and *Scaptia* and some primitive chrysopines and tabanines have precisely that distribution. What appear to be modern Tabaninae and possibly Chrysopinae were established by the Oligocene (Cockerell 1921; Statz 1940) and Miocene (Cockerell 1909, 1916; Melander 1946).

We may imagine the ancestral Tabanidae, then, as developing about the beginning of the Mesozoic. They were probably low-flying species, of medium to rather slender build, not unlike existing *Ectenopsis,* or primitive Chiromyzinae, or the primitive nemestrinid *Exeretoneura*; and they probably fed on the juices of plants. They had bare eyes, holoptic in the male; ocelli; a diverging, tomentose frons in the female; short antennae, with the third segment subulate and eight-annulate; a short, stout proboscis, with well-developed, soft labella; well-developed mandibles in the female; and subcylindrical or somewhat flattened, slender palpi. The legs had spurs on mid and hind tibiae,

but none on fore; the wings had the costa circumambient, basicosta and vein *sc* bare, R_4 with a well-developed appendix (possibly pectinate on R_{2+3}), cell R_4 short and wide, and cells R_5 and M_3 open; squamae were moderately developed. The ninth tergite was entire in both sexes, shield-like in the male; the aedeagus a smooth, conical tube; style simple; eighth sternite of female shield-like, with prominent, rounded gonopophyses; cerci one-segmented; caudal ends of spermathecal ducts simple, lightly reinforced tubes.

The first major cleavage in this ancestral population (or group of populations) came with the splitting and reduction of the ninth tergite, initially in the males, later in the females also. It is probable that the general trend in the Tabanoidea towards shortening of the abdomen by retraction of the apical segments had already appeared, and the smaller, more flexible tergite may have had advantages in relation to it. The more primitive element with an entire tergite nevertheless survived and developed into the modern Pangoniinae, which, I believe, have had their own separate evolutionary history from that ancient beginning.

The Scepsidinae, from the slender available evidence, appear to have developed as an offshoot from the base of the second line of evolution, before the tergite split in the female or reduction in the annuli of the third antennal segment became apparent. They have retained a primitive, somewhat therevid-like facies, but undergone some remarkable specializations of their own.

Four main trends then appeared. The first was an adaptation to blood-sucking, leading to increased compactness of the body, but retention of the relatively short, strong proboscis and legs. The second was an adaptation to living on nectar, leading to stouter build, longer legs for clinging to flowers, and a longer, more slender proboscis, often with reduced labella, and sometimes associated with a snout-like prolongation of the face. It is probable that the ancestors of the Chrysopinae and Tabaninae became associated mainly with the vertebrates, those of the Pangoniinae mainly with the ancestors of the flowering plants, but that both associations developed independently more than once in the family, as they did in related families (for example, blood-sucking in the rhagionid genera *Spaniopsis* and *Sympharomyia*, flower-haunting in Pelecorhynchidae and Nemestrinidae), and one may have given place to the other in the evolution of individual genera. In modern Pangoniinae, some females feed only on blood, some only on nectar, and some on both.

The third trend was an important one, common in the Diptera, a tendency to reduction with increasing specialization. It is seen here particularly in compaction of the third antennal segment, in the loss of hind tibial spurs and ocelli, which led to the separation of Tabaninae from Chrysopinae, and in a more diffuse tendency for wing veins to fuse, producing closed marginal cells.

The fourth trend is seen in the evolution of the primitive, soft-bodied, semi-terrestrial pangoniine larva, living in damp soil or mud and feeding on other soft-bodied creatures, such as earthworms, into the stronger, torpedo-shaped, more vigorously predaceous larvae of Chrysopinae and Tabaninae, which attack their own kind or anything else offered to them, and are adapted to a wide

range of environments from open ponds to rot-holes in trees. Stigmatal specializations associated with an aquatic life seem to have appeared earlier in the Chrysopinae than the Tabaninae.

There have been other adaptations, too, for example, to mimicry, to breeding in tree-holes, to life on the sea-shore, but these have affected smaller groups, although their influence on morphology and behaviour has sometimes been considerable.

One would have expected that an evolution of the kind described, complete in main essentials before the middle of the Mesozoic, would have led to a stable classification, but there have been several complicating factors. The first is that the trends described have been truly trends. Species have, so to speak, dropped off all along the way, and one is faced with continuous series linking genus with genus and tribe with tribe. These certainly belong to later evolution, but even the ninth tergite is not quite constant, being incompletely divided (or secondarily fused) in the males of a few Bouvieromyiini, and attenuated to breaking point in the middle in the females of a few Philolichini. Fortunately, in both instances, the other characters of the insects decide their position unequivocally.

The second factor is the failure of isolation to lead to the production of secondary characters of taxonomic value. The genitalic differences described probably belong to this class. Their reliability seems to be complete; but they are useful only to distinguish the subfamilies and the tribes of Pangoniinae, which are decidedly ancient divisions. The later development of setulae on the basicosta of some Tabaninae is another example, and it would be difficult to cite any more.

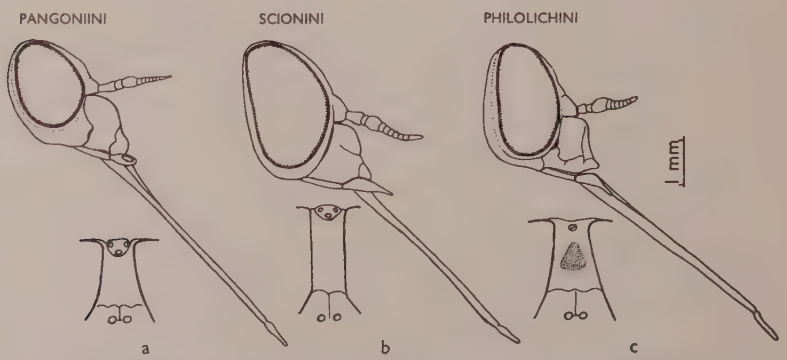


Fig. 5.—Pangoniinae. Representatives of the three tribes, showing resemblances due to convergence. *a*, *Pangonius mauretanus* var. *aethiops*; *b*, *Scione* sp. nr. *maculipennis* (Schin.), S. America; *c*, *Dorcaloemus compactus* Aust., Africa.

A third factor, which is of great importance, because it has been frequent in this family, is convergence, due to parallel adaptation to a common mode of life. It can be illustrated rather strikingly (Fig. 5) by the heads of representatives of each of the three tribes of Pangoniinae. Hairiness of the eyes

is relatively reliable in Pangoniinae, but is so frequently associated with life in a cool climate in Chrysopinae and Tabaninae that it has little phylogenetic value in those subfamilies. The independent evolution of a laterally compressed tip to the female abdomen, with a narrow eighth sternite, at least four times (in Rhinomyzini, once in Diachlorini, and at least twice in Tabanini) is probably another example, for Oldroyd (personal communication) has suggested that it may be an adaptation to ovipositing in tree-holes or other unusual situations.

There is another phenomenon, which may be conveniently grouped with convergence, for it is often difficult to distinguish between the two, and that is a tendency for characters which are not so obviously adaptive to appear more than once in the family. This is not surprising when they represent a general trend, as in fusion of the basal annuli of the third antennal segment in some Pangoniini as well as in all Chrysopinae and Tabaninae, or the independent closure of cell R_5 in various members in all subfamilies; but other cases are more difficult to explain, as for example, the parallel modifications of the third antennal segment in Chrysopinae and Tabaninae illustrated in Figure 4, or the fact that a long dorsal process on the third antennal segment has evolved independently at least eight times in these two subfamilies. Nicholson (1927) has suggested an inherent capacity in the common genetic material of the group to explain the development of metallic coloration in a number of different Diptera (it has appeared several times in the Tabanidae), and some such explanation may account for the phenomenon noted here.

The final complication is presented by the occasional disappearance of a character which is otherwise firmly established. Thus, there is no doubt that the abundant setulae on basicosta characteristic of Tabanini are a fairly recent acquisition; yet a few Palaearctic species lack setulae, and must be presumed to have lost them, as they differ from their immediate local relatives in no other important respect.

It is little wonder that the Tabanidae have been a difficult family to classify, and that their zoogeography has been confused.

Subfamily Pangoniinae

The distribution of the subfamily as a whole is almost world-wide, as might be expected of such an ancient group, but important differences appear when the tribes are examined separately (Fig. 6).

Tribe Pangoniini

This tribe contains genera which are in many ways the most primitive in the family, although all have a specialized style in the male hypopygium, and one group shows marked advances in specialization parallel to those seen in the other tribes. It is noteworthy that some of the genera included here have the only form of palpi in the subfamily which could have been ancestral to the type found in Chrysopinae and Tabaninae. Some of these show, too, a reduction in the annuli of the third antennal segment (Fig. 4) parallel to that seen in the higher subfamilies. It is not suggested that these are on the direct line

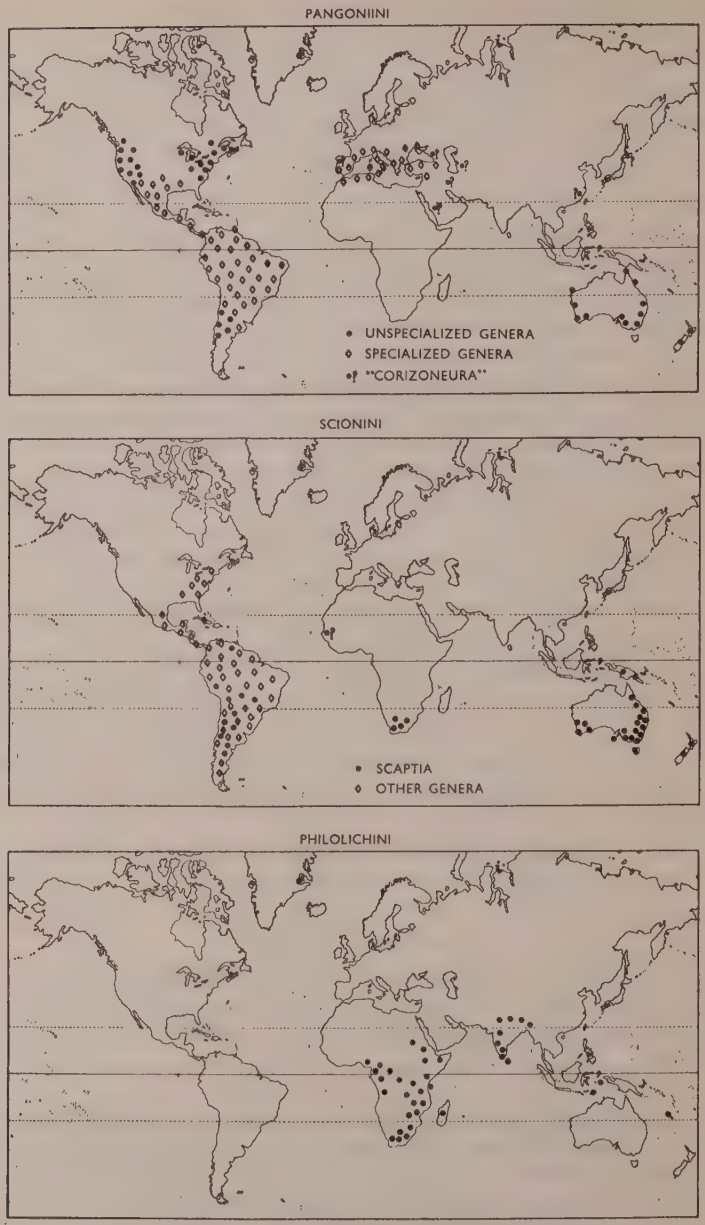


Fig. 6.—Distribution of Pangoniinae.

of evolution of the other subfamilies, but that an ancestral form with a simple male style was common to both.

The less specialized element is found in the Nearctic, southern Neotropical, and Australasian regions, including New Zealand* and the Torres Strait islands, but not Tasmania, New Guinea, or the Austro-Malayan area. It is not known from the Ethiopian or Oriental regions, nor from Oceania east of New Zealand. The question of its occurrence in the Palaearctic region cannot yet be answered. *Scaptiella aperta* (Loew) from Portugal might be a scionine, and so possibly might several species of "Corizoneura" described from southern Europe, the Caucasus, Trans-Caspian, China, and Japan, but it seems more likely that they are all generalized Pangoniini related to the Nearctic *Pilimas* and *Stonemyia.* Re-examination of these species, and particularly dissection of the males, is highly desirable, because they are of considerable zoogeographical importance.

The more specialized genera are found only in the Palaearctic, Nearctic, and Neotropical regions. Their greatest development is in Central and tropical South America, and they disappear in the far south.

Tribe Scionini

This tribe is the least specialized in genitalic characters, but more specialized than generalized Pangoniini in adaptations both to flower-haunting and blood-sucking habits. There are two bare-eyed, aberrant genera, the Nearctic *Goniops* and the southern Neotropical *Mycteromyia,* the latter so extraordinary in hypopygial characters that it almost warrants a tribe to itself. The remaining genera all have hairy eyes, and they form a single progressive series. The separation between generalized species of *Scaptia* with short proboscis and long palpi (*aurata* group, Fig. 2) and those with long proboscis and short palpi (*maculiventris* group, Fig. 7) is not clearly defined in Australia, but apparently sharper in South America, where the latter (under the names *Listrapha, Parosca,* and *Listriosca*) lead through *Pseudoscione* directly to highly specialized *Fidena* with prominent snout, reduced labella, and sometimes extraordinarily long proboscis. *Scione,* with cells R_5 and M_3 both closed, and *Pityocera,* with bizarre modification of the third antennal segment, are offshoots from the same sequence.

Scionini are represented in the Nearctic region by a single monotypic genus. They are highly developed and varied in the Neotropical region; represented by a few species of *Scaptia* in the southern part of the Ethiopian region; and strongly represented in the Australasian region by 64 species of *Scaptia* and near relatives in Australia, 12 in New Guinea, and five in New Zealand. I have been able to compare specimens of *Scaptia* from Chile, South Africa, and the three parts of the Australasian region, and can confirm their generic identity, the *maculiventris* group (Fig. 7) being common to all the areas, the *aurata* group (Fig. 2) common to Chile and Australia, and the remaining groups local.

There may be one doubtful representative (*Scaptiella,* referred to earlier) in the Palaearctic region, but none is known from the Oriental region.

* *Apatolestes lutulentus* Hutton links the Australian genera *Ectenopsis* and *Parasilvius.*

Tribe Philolichini

This tribe evidently had a common origin with the Scionini, and its genera can be arranged in a similar sequence, thus: *Buplex* → *Ommatiosteres* → *Nuceria* → *Philoliche*. However, they all carry distinctive markers in the shape of the eighth sternite of the female, and to a significant, though not so complete, degree in bare eyes, loss of ocelli, and presence of a strong appendix on R_4, so it is clear that we are dealing with two parallel lines and not a single evolutionary development. Nevertheless, the superficial correspondence is remarkable, for example, between *Fidena* and *Philoliche*, and between the respective offshoots *Scione* and *Dorcaloemus*. The other offshoots, *Pityocera* and its allies on the one side and *Phara* and *Subpangonia* on the other, have developed on quite different lines. There is no mixing of the more specialized faunas, and it seems evident that the South American and African radiations took place independently, and therefore later than the evolution of *Scaptia* and the more primitive tribes of Chrysopinae and Tabaninae.

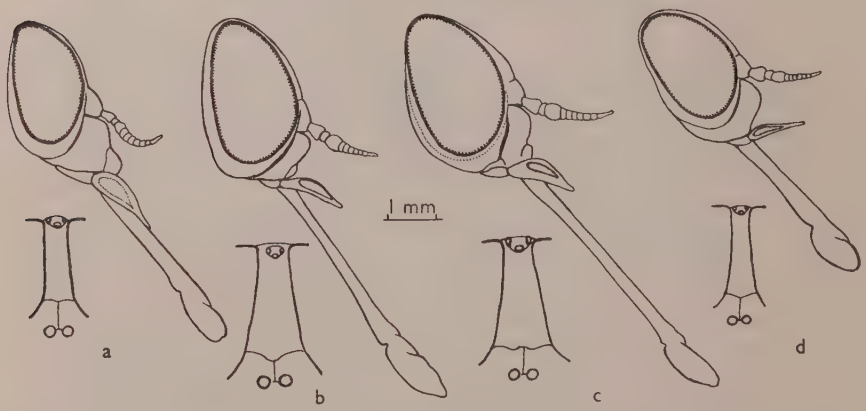


Fig. 7.—Heads of species of *Scaptia* from different countries. *a*, S. *viridiventris* (Macq.), Chile; *b*, S. *barbata*, S. Africa; *c*, S. *maculiventris* (Westw.), Australia; *d*, S. *adrel* (Walk.), New Zealand.

Philolichini are almost exclusively Ethiopian. There may, however, be a small extension into the southern part of the Palaearctic region (Kröber 1939), and there is a definite Oriental extension represented by three or four species of *Nuceria* in India and Ceylon, while another three species of *Nuceria* form a detached element in the northern part of the Australasian region in Amboina, Timor, and New Caledonia. It seems possible that some of the gaps in the eastern distribution may be bridged by further collecting.

Subfamily Scepsidinae

This small group includes only four monotypic genera, which are adapted to life on the sea-shore or other sandy areas. It is intermediate between Pan-

goniinae and Chrysopinae in so many respects that it cannot be placed readily with either, and the extraordinarily reduced mouth-parts and modified female terminalia set it apart from both.

One genus is Neotropical (Brazil), and three are Ethiopian (east coast of Africa from French Somaliland to the Cape of Good Hope).

Subfamily Chrysopinae

The distribution of Chrysopinae (Fig. 9) is similar to that of Pangoniinae, but with the significant difference that none is known from Tasmania or New Zealand. There is another difference, in that a quality of invasiveness has appeared. If the relatively modern, vigorous genus *Chrysops* were removed, the basic zoogeography of the two subfamilies would be astonishingly alike.

Fig. 8.—Distribution of Scepsidinae.

Tribe Bouvieromyiini

This is the most ancient element in the subfamily, and most of the genera have remained quite generalized, although there have been centres of more vigorous generic evolution in Africa and Australia.

It is represented in the Nearctic region by a single genus (*Merycomyia*), and in the southern part of the Neotropical region by several species of *Veprius*; it is well developed and vigorous in the Ethiopian region; and it is also vigorous in Australia, with a northern extension into New Guinea and possibly a few of the Austro-Malayan islands. The only Oriental representative, *Eucompsa*, from Java and Borneo, is evidently derived from the Australian *Pseudotabanus*. There may also be a small Palaearctic extension represented by two Mediterranean species of *Mesomyia* (Kröber 1939).

The two features of special interest about the distribution are: firstly, the extremely wide gap in tropical America separating the northern temperate *Merycomyia* from the southern temperate *Veprius*, and the parallel emptiness of the Oriental region; and secondly, the strong South American-African-

Australian relationship. Comparison of African *Mesomyia,* Chilean *Veprius,* and the Australian *Pseudotabanus* and *Lilaea* leaves one in doubt whether even subgeneric distinctions between them can be maintained. *Lilaea* has some distinguishing features; but *Pseudotabanus* can be divided into two sections, the bare-eyed species merging into *Veprius* and the hairy-eyed ones into *Mesomyia.*

Tribe Chrysopini

This tribe appears to be a relatively recent derivative from primitive Bouvieromyiini, from which it is only moderately well differentiated. It is predominantly Holarctic in distribution, and has undergone its greatest radiation there, with three genera (*Silvius, Nemorius,* and *Chrysops*) in the Palaearctic region, and four (*Silvius, Chrysops, Neochrysops,* and *Assipala*) in the Nearctic, the last being an extension of a small northern Neotropical development. It is doubtful whether *Silvius,* in the restricted sense, occurs in the Ethiopian region; but one species of *Silvius* and *Melissomorpha indiana* Ric. (which probably belongs here) have been recorded from the extreme north of the Oriental region.

There are so many intermediates between *Silvius* and *Chrysops* in the Nearctic region, that it is tempting to think that *Chrysops* developed there. It certainly seems to have radiated from the north, there being about 60 Palaearctic species, 73 Nearctic, 65 Neotropical (of which only four are Chilean), 40 Ethiopian, only 19 Oriental, 3 Austro-Malayan, and a single Australian species in north Queensland. This distribution agrees perfectly with Matthew's (1915) hypothesis of radial dispersal, which was based on eutherian mammals, and it seems reasonable to suggest that *Chrysops* may have evolved and dispersed with them.

Tribe Rhinomyzini

Africa has been the centre of three evolutionary bursts in the family, the Philolichini, a minor one in the Bouvieromyiini, and this, which is the most vigorous of the three. There are indications in its most generalized member, *Tabanocella,* that it originated from primitive Bouvieromyiini, but many of its members show quite extraordinary responses to life in the wet tropics and probably to a habit of breeding in rot-holes in trees.

The distribution is broadly similar to that of the Philolichini, but more restricted, with most genera in tropical Africa and the Malagasy subregion, and an Oriental extension represented by five species of *Gastroxides* in India, Ceylon, Malaya, and east China, and two of *Rhinomyza* in Java.

Subfamily Tabaninae

Generalized Chrysopinae and Tabaninae (e.g. *Pseudotabanus* and *Cydistomyia,* or *Mesomyia* and *Dasybasis*) resemble each other closely, and, indeed, can only be distinguished with certainty by the small but essential subfamily characters of the male and female genitalia, and somewhat less reliably by the hind tibial spurs and ocelli. It seems, therefore, that they did not diverge from one another until the Chrysopinae had been separated from their pan-

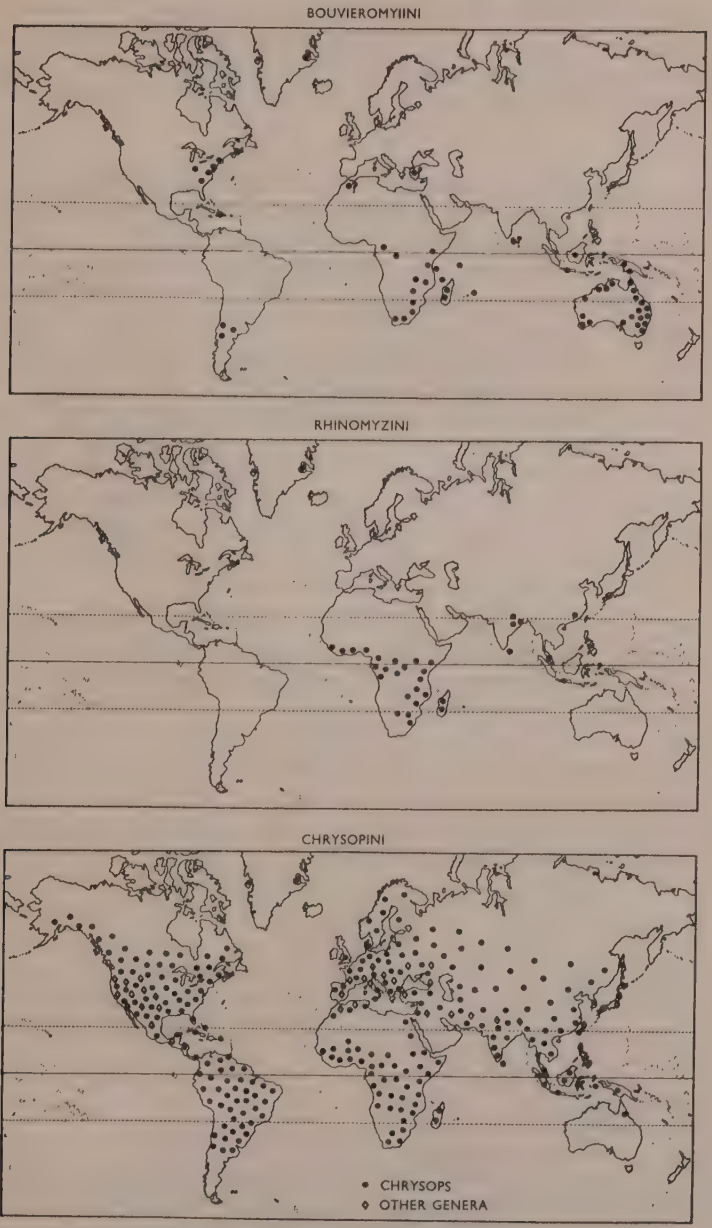


Fig. 9.—Distribution of Chrysopinae.

goniine ancestors sufficiently long for their characteristic facies to have become firmly established. The only justifications for giving them more than tribal rank

are that the cleavage must have been ancient, and that it is convenient to provide for further subdivision, particularly in the Tabaninae, which contains more species than the other subfamilies together, and has a distribution over the whole world, including several islands of the Pacific Ocean (Fig. 10).

Tribe Diachlorini

The more generalized members of this tribe correspond with the Bouviero-myiini in the Chrysopinae, and they have an essentially similar distribution. They are doubtfully represented in the Palaearctic region by a very small number of species. There are about 10 Nearctic species, most of which form a Neotropical element in the fauna. The richest development of the tribe is in the Neotropical region, where there are many species, which have been divided among several difficult "genera," which are quite distinct at their extremes, but merge imperceptibly into one another. There is a similar, but smaller, element in the Ethiopian region, of which Oldroyd (personal communication) writes: "I recognize five genera . . . (which) occur only in southern and south-eastern Africa, the eastern littoral and the islands of the Malagasy area . . . I have made several observations, such as you make in your letter, how similar these genera are to the Silviines on the one hand and to some *Tabanus* on the other; . . . and how they seem, both geographically and structurally, to link with South America and Australasia."

In the Australasian region, this group is the dominant element in the Tabanine fauna, and presents the same difficulty in intergrading "genera" that was noted for South America. There are approximately 90 species in Australia, 25 in New Guinea and the adjacent islands, 12 in New Zealand, and three in Fiji. There is probably also a small extension of *Cydistomyia* west of Weber's line, but the only other Oriental representative of the group appears to be *Neotabanus ceylonicus* Ric. from Ceylon, and it is somewhat specialized.

The specialized groups in the tribe have a more restricted distribution. Most of them belong to an intense Neotropical radiation (with a small Nearctic extension of two species), parallel, and probably contemporaneous, with the radiation of Rhinomyzini in the Ethiopian region. On the other side of the world, there are independent Oriental-Australian elements represented by *Udenocera brunnea* Ric. in Ceylon, two species of *Lissimas* in Java and Celebes, three of *Neobolbodimyia* in the Celebes, Aru Is., and New Guinea (possibly one also in Fiji), and *Paracanthocera australis* (Ric.) in north Queensland.

Tribe Haematopotini

There is evidence to suggest that this tribe arose from *Dasybasis*-like ancestors with wide, diverging frons and larvae without siphons, by elongation and thickening of the antennae, reduction of the antennal style to three annuli, appearance of a few setulae on basicosta, and development of characteristic wing markings. It is probably of comparatively recent origin, for its distribution is like that of *Chrysops* though more restricted. There are five Nearctic species of *Haematopota*, 53 Palaearctic (plus one *Heptatoma*), 171 Ethiopian

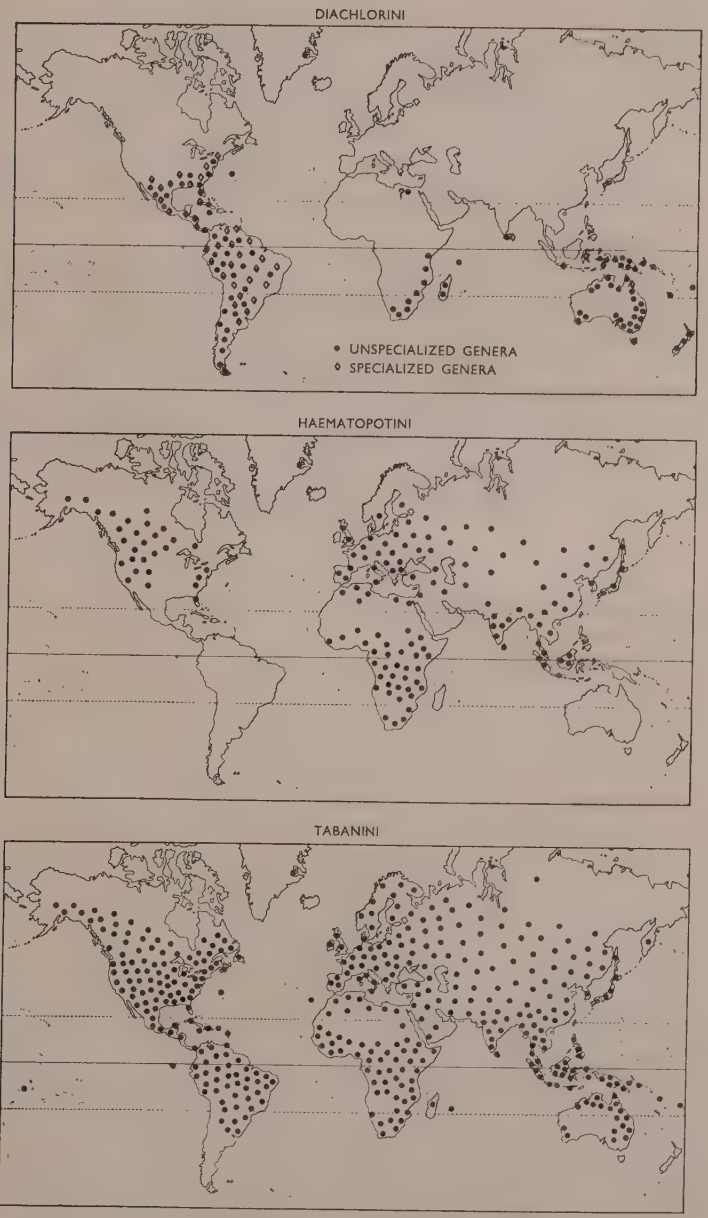


Fig. 10.—Distribution of Tabaninae.

(plus three *Hippocentrum*), and 65 Oriental, the distribution ending at Java. The tribe is not known from the Australasian or Neotropical regions.

Tribe Tabanini

This highly successful group of about 1000 species is the evolutionary climax of the family. It merges into the Diachlorini in the Neotropical region, but elsewhere it is well defined, compact, and uniform. Such diversity as there is appears to be greatest in the Palaearctic and Nearctic regions, where a number of groups of related species have been given generic rank. There are only a few really aberrant forms, such as the Ethiopian *Ancala* and *Euancala,* though the latter is distinguished more by striking coloration and markings than by structural features.

The tribe has radiated widely, and is abundantly represented by something between 150 and 300 species in each of the Palaearctic, Nearctic, Neotropical, Ethiopian, and Oriental regions. The number decreases rapidly in the southern part of South America (Fairchild, personal communication), and is also considerably reduced in the Australasian region, where the tribe seems to be a recent invader from the north, there being about 20 species in the Austro-Malayan islands, 32 in New Guinea, and 20 on the mainland of Australia. None is known from Tasmania or New Zealand, but I have seen a species of *Tabanus* from Fiji, and one has been described from Samoa.

IV. Conclusion

From the foregoing, the probable evolutionary history of the Tabanidae may be summarized, as a working hypothesis, broadly in the following terms. The family developed as an independent unit early in the Mesozoic. Initial evolution was fairly rapid, and the four subfamilies, more primitive tribes, and even some well-defined genera, such as *Scaptia,* were established by the middle of the Mesozoic. Then followed a long period of quiescence, broken by vigorous Tertiary evolution of the more specialized tribes and genera. Finally, there has been another, quite recent, possibly Pleistocene, burst of evolutionary activity with separation of groups, which are imperfectly differentiated, and may be termed incipient or embryonic genera. An essential part of this last has been vigorous speciation and subspeciation in many parts of the world, but a discussion of this is beyond the scope of the present paper.

Whether this hypothetical evolution proves to be correct in detail or not, it is clear that there are three basic zoogeographical patterns in the family, and that groups in corresponding stages of development in the different subfamilies show the same distributional pattern. The pattern of the more primitive elements (Pangoniini, Scionini, Bouvieromyiini, unspecialized Diachlorini) is essentially "Gondwanaland" or "Antarctic," with the Pangoniini showing a strong Holarctic arc similar to that of the subgenus *Ochlerotatus* in the Culicidae. More specialized elements show two main patterns. One is regional radiation in the south, with limited extension, shown by Scionini and specialized Diachlorini in South America, Philolichini and Rhinomyzini in Africa, and the maps indicate how similar are their respective pathways of extension. The other is the typical Holarctic distribution and radial dispersal of Matthew, shown by *Chrysops,* Haematopotini, and Tabanini. *Haematopota,* incidentally, is the only large genus in the family that respects Wallace's line.

In all these respects, the Tabanidae present a series of examples of zoogeographical phenomena which are characteristic of many of the older groups of animals. They conform, in fact, to patterns which strongly suggest that there has been a major change in available pathways of dispersal in the course of evolutionary history. That the one group should supply so many parallel instances supporting the hypothesis seems to me to be particularly significant, but it would be unwise to say more until some of the vital question marks on the maps can be removed.

V. Acknowledgments

This work is based, in the first instance, on an examination of most of the collections of Tabanidae in Australia and New Zealand, and separate acknowledgments to the many individuals and institutions concerned are given in the more detailed paper which is in preparation. The wider survey, however, would not have been possible without the generosity of Dr. G. B. Fairchild, Gorgas Memorial Institute, Panama; Mr. H. Oldroyd, British Museum (Natural History); and Dr. Cornelius B. Philip, Rocky Mountain Laboratory, Montana, U.S.A.; who gave or loaned to me named representatives of most of the major genera from other faunal regions, and who also contributed many ideas in the course of long and stimulating correspondence.

In addition to my great indebtedness to several colleagues for material and information, I would like to express my thanks to my wife for a great deal of help with the illustrations.

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Corrigenda

Volume 1, Number 2

Page 271: *For* 8. Trichophthalma thomsoni, sp. nov. ♂ *read* 8. Trichophthalma thomsoni, nom. nov. ♂.

Volume 2, Number 2

Page 291: Fig. 4.—Hatched bars refer to cattle-type larvae and unhatched bars to sheep-type larvae.

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